THIS NUMBER COMPLETES VOLUME 18

DECEMBER 10, 1939

THE JOURNAL OF NUTRITION

VOLUME 18 NUMBER 6

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Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign; Two volumes issued annually

Entered as second-class matter January 20, 1934, at the post office at Philadelphia, Pa., under Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917, authorized on July 2, 1918

OFF PRESS DECEMBER 20th!

1939 INDEX

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in

• ANATOMY • ANTHROPOLOGY • MORPHOLOGY

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THE USE OF ISOLATED RADIATION IN EXPERIMENTS WITH THE RAT

II. EFFECTS OF DARKNESS, VISIBLE, AND INFRA RED RADIATION ON THREE SUCCEEDING GENERATIONS OF RATS

(*a*) GROWTH AND STORAGE OF VITAMIN A[1]

ETHEL M. LUCE-CLAUSEN AND ELIZABETH F. BROWN
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THREE FIGURES

(Received for publication July 31, 1939)

INTRODUCTION

Jones ('29) has described a series of filters for the isolation of spectral bands in the ultra-violet, visible and infra red regions of the spectrum. A report of experiments made with the use of these filters has also been published (Luce-Clausen, '29). As an outcome of this work a series of four rooms was designed to provide a radiation environment in which rats could be studied, in suitable numbers, over long periods of time. A description of these rooms, with data on wave length limits and energy values of radiation, has been given by Jones and Tuttle ('39). The rooms will be designated:

Room I—Complete darkness
Room II—Near infra red radiation
Room III—Visible radiation
Room IV—Far infra red radiation

Experiments were started in September, 1936, and were concluded in the spring of 1938. Studies were made of growth, storage of vitamin A, and of reproduction. This paper reports results on growth and storage of vitamin A.

[1] Aided by a grant from the Committee on the Effects of Radiation upon Living Organisms, Divisions of Biology and Agriculture, National Research Council.

EXPERIMENTAL PROCEDURE

At the beginning of the experiment sixteen pregnant females of the Experimental Colony strain, were shipped to us from The Wistar Institute. These rats were, within the limit of 4 days, of the same age; one group of five and another of six were litter mates. We distributed them, four in a room, sampling them so that there were, as far as possible, litter-mate rats from the sixteen, in each room. The mothers, after the young were weaned, were discarded. The young were raised in the different rooms and bred through three generations.

The rats were housed on tables placed immediately under the duct which carried the radiation equipment in each room. We tried, as far as possible, to eliminate all radiation from the dark room. It contained no radiator. The outlet duct from the radiation rooms (see diagram Jones and Tuttle) was in the dark room, but was well insulated, never more than slightly warm to the touch, and situated in a part of the room remote from the rat cages. The rats were radiated for 12 hours each day, 5 A.M. to 5 P.M., the lamps being turned on and off automatically by the use of a Sangamo time switch.

Rats were weaned on the twenty-eighth day. The original mothers were fed the Sherman B diet (Sherman and Campbell, '24) plus 10% meat scrap until the young were weaned. The young were then divided, in each room, into two groups. One group was reared on the Sherman B, the other on the 'McCollum' diet (Evans and Bishop, '22). We used the Sherman B diet because it was the one used in other experiments. The McCollum diet was chosen as being the best and most standard diet for studies on reproduction. The animals were fed ad libitum.

Unfortunately the commercial casein used in the 'McCollum' diet was found, after a period of a year, to be contaminated with fluorine. We have published (Hodge, Luce-Clausen and Brown, '39) an account of the teeth of the rats in this group and indicated, as a result of this accident, our findings as to the effect of darkness on fluorosis.

In analyzing results we have taken the presence of fluorosis into account, and have given results on each diet separately. The 'McCollum' diet has been indicated in quotation marks to signify the diet plus its contamination.

As a routine procedure all mother rats were fed a total of 1300 units of vitamin A in Haliver oil during lactation.

Records of daily growth during lactation, and of weekly growth after weaning were kept.

RESULTS

Growth (fig. 1)

The points on the curves represent mean weights for varying numbers of rats as shown in table 1. The numbers given are those at the beginning and at the end of the experiments. They diminish because some of the rats were killed on the seventeenth and fifty-fourth days to permit determination of the amounts of stored vitamin A; also only males needed for breeding were kept. Although the number used for the adult curves is not large, the results are very consistent.

The upper curves (fig. 1) represent early growth from birth to weaning. The lower set show the growth records of male rats up to adult life. In all cases the results in the two infra red rooms were so nearly alike that they have been combined in one curve. Lack of space prevents us from publishing all our growth curves. Those included demonstrate certain tendencies with which we have been impressed.

1. An effect of radiation is indicated because the same order is maintained throughout, namely, the curves for rats deprived of light show the slowest rate of growth (I), those for rats receiving visual radiation a faster rate (III), while the results for rats exposed to infra red radiation, in every case, are intermediate.

2. Sherman B rats. The difference in rate of growth between the various groups is first indicated in the second generation, beginning at about the fifth day and being maintained throughout the 15 weeks of observation. In the third genera-

tion this divergence is increased, partly due to a progressive decline of the growth rate of the rats in room I and partly due to a progressive improvement of growth of those in room III.

3. For rats on the 'McCollum' diet we see similar but intensified divergences. These are due mainly to failure of growth of the rats in room I, and maintenance of growth of those in room III. The early growth of the rats in room I is strikingly lower than that of the second and third generation rats fed

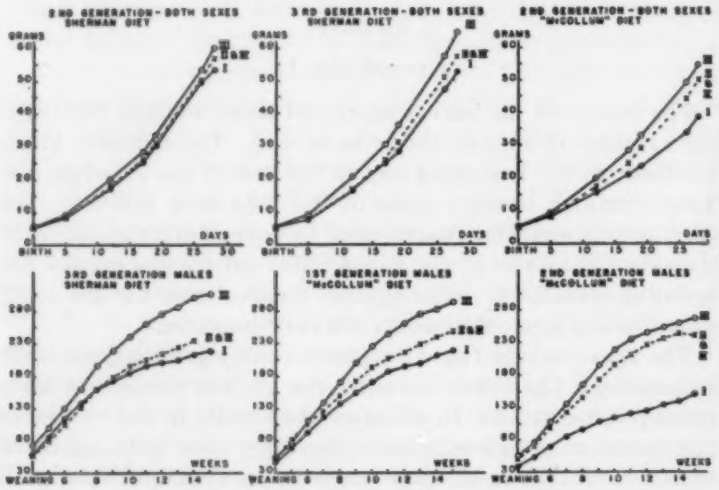

Fig. 1 Growth. I—rats in complete darkness. II and IV—rats exposed to infra red radiation. III—rats exposed to visible radiation.

the Sherman B diet. We attribute this to the effect of fluorine in the diet. Fluorine has not affected the early growth of the radiated animals to any great extent.

The later growth of the first generation male rats is strikingly similar to that of the third generation male rats on the Sherman B diet. In the second generation the radiated rats on this diet grew nearly as well as they did in the first generation, although there was a marked failure of growth in the rats in room I. Growth was maintained by the radiated animals even in the third generation.

4. Our results on female rats, which are not included, indicate a definite but less potent effect of radiation on the growth of female than on the growth of male animals.

TABLE 1

Number of rats used for growth curves[1]

ROOMS	BIRTH TO WEANING SHERMAN DIET 2ND GENERATION	BIRTH TO WEANING SHERMAN DIET 3RD GENERATION	BIRTH TO WEANING 'MCCOLLUM' DIET 2ND GENERATION
I	34–18	24–15	70–31
II and IV	67–36	68–27	152–58
III	42–25	27–14	40–24
	ADULT MALES SHERMAN DIET 3RD GENERATION	ADULT MALES 'MCCOLLUM' DIET 1ST GENERATION	ADULT MALES 'MCCOLLUM' DIET 2ND GENERATION
I	5–3	9–9	16–4
II and IV	9–4	15–9	25–7
III	5–2	8–4	8–5

[1] The numbers given are those at the beginning and at the end of the experiments (see text).

Vitamin A storage

Representative rats from all the rooms, on both diets, were killed on the seventeenth and fifty-fourth days and their livers analyzed for vitamin A. Similar analyses were made of all adult rats at autopsy. The method used for the vitamin A determinations was the modification of the Price-Carr method of S. W. Clausen (McCoord and Luce-Clausen, '34).

Figure 2 gives the units [2] of vitamin A in the liver plotted against the weight of the rat, with respect to both sexes at 17 days, males at 54 days, and adult males in all the groups. Figure 3 gives results for females.

In figures 2 and 3 the position of the line for each group of rats was determined by the method of least squares.

It is evident that in plotting the vitamin A values at given ages, the weight of the rat should be taken into account since these lines show a general upward slope with increased weight, at any given age. In cases, however, where we fed supple-

[2] One of our units = 3.8 international units.

ments of vitamin A to mother rats during lactation, this relationship of storage to body weight did not hold. The vitamin A values given in figures 2 and 3 were obtained from rats to whom no supplement had been fed.

The rats used in these determinations were taken from all three generations, but predominantly from the second and third.

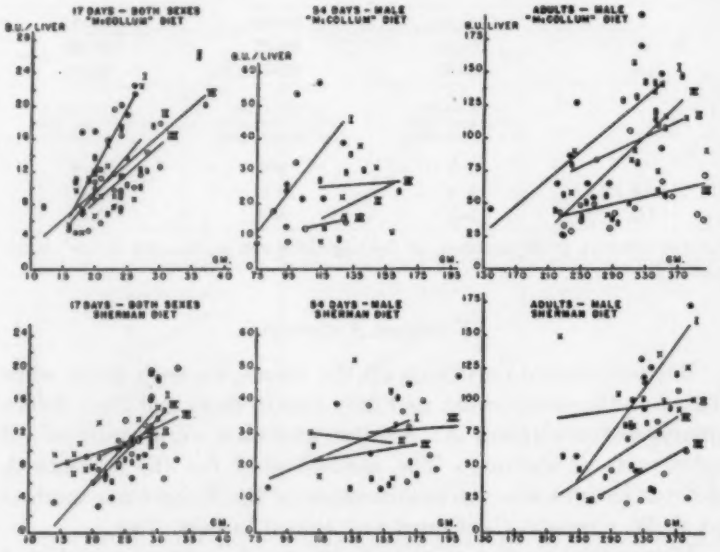
17 DAYS – BOTH SEXES "McCOLLUM" DIET
54 DAYS – MALE "McCOLLUM" DIET
ADULTS – MALE "McCOLLUM" DIET
17 DAYS – BOTH SEXES SHERMAN DIET
54 DAYS – MALE SHERMAN DIET
ADULTS – MALE SHERMAN DIET
S.U./LIVER
GM.

Fig. 2 Vitamin A in livers plotted against body weight. I—rats in complete darkness. II and IV—rats exposed to infra red radiation. III—rats exposed to visible radiation.

In considering the trend of these lines we were struck by the fact (fig. 2) that in six separate groups of observations the rate of increase of vitamin A with weight is greater with the animals in room I. The line for adult males in room IV ('McCollum' diet) runs parallel with that of room I, but the amount of vitamin storage, for any given weight, is greater in room I.

In figure 3 (females) the lines for rooms II and IV are similar to those of room I, but all are widely different from that of room III.

We decided therefore to compare the extremes, rooms I and III, which represent the wide difference between an environment of total darkness and one of visible radiation. Although at first sight, in view of the wide variations that are normally obtained in determinations of vitamin A, the difference in

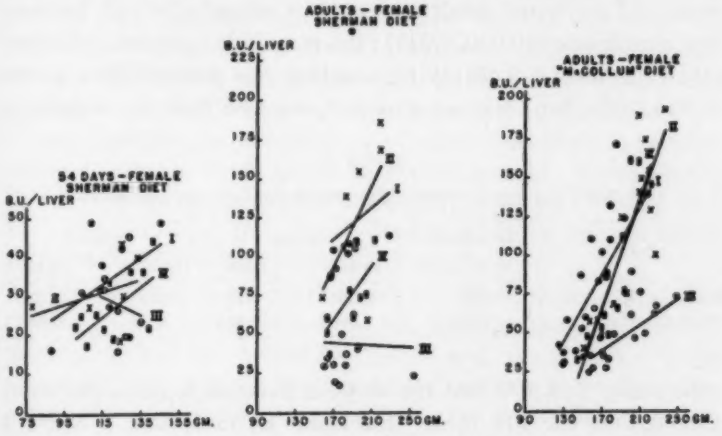

Fig. 3 Vitamin A in livers plotted against body weight. I—rats in complete darkness. II and IV—rats exposed to infra red radiation. III—rats exposed to visible radiation.

values for room I as compared with those for room III might not seem significant, the regularity with which they appeared in nine different groups of observations made further statistical analysis seem desirable. The method of analysis was as follows. The slope of each line was designated (b). The standard error (ε_b) was calculated for each slope.[3] The standard error of the differences of the slopes [4] for rooms I and III ($\varepsilon_{b_I - b_{III}}$)

[3] $\varepsilon_b = \sqrt{\frac{1}{(N-2)}\left[\frac{N\Sigma y^2 - \Sigma^2 y}{N\Sigma x^2 - \Sigma^2 x} - b^2\right]}$

y = ordinate; x = abscissa; b = slope.

[4] $\varepsilon^2_{b_I - b_{III}} = \frac{(N_I - 2)\,\varepsilon^2_I}{(N_{III} - 2)} + \frac{(N_{III} - 2)\,\varepsilon^2_{III}}{(N_I - 2)}$

was then calculated. The ratio of the difference between slope I and slope III over the standard error of the difference was then found. The probability of obtaining a ratio as large as, or larger than this, by pure chance was then determined by 'Student's' t table.[5]

Results as analyzed from figures 2 and 3 are given in table 2. It is seen that in one case (Adult females, 'McCollum' diet) the difference in slopes is clearly significant (0.008); in two cases (17 days and adult males, 'McCollum' diet) of borderline significance (0.025, 0.025); the remainder are not individually significant. Multiplying together the probabilities given in the table, for each sex and diet, we find that the chance is

TABLE 2

Probabilities that observed differences in slope are not significant

DIET	BOTH SEXES	MALES		FEMALES	
	17 days	54 days	Adult	54 days	Adult
Sherman B	0.10	0.35	0.10	0.15	0.25
'McCollum'	0.025	0.30	0.025	...	0.008

well under 1 in 100 that the slope difference is not significant throughout the life cycle (Sherman B, male and female, 4 chances in 1000; 'McCollum,' male and female, 2 chances in 10,000). Therefore in each case there is a significant slope difference at some one or more stages of the life cycle. It would seem therefore statistically justified to consider the different values for room I as compared with room III as not being chance variations within both groups, but as true differences in rates of increase of vitamin A, at given ages, in the absence or presence of radiation in the rats selected.

In this analysis we note, what we have found in many of our analyses, that the presence of fluorine in the diet intensifies an effect produced in rats fed the uncontaminated diet.

[5] "Statistical Methods for Research Workers" by R. A. Fisher, 7th edition, published by Oliver and Boyd, Edinburgh, 1938, p. 177.

DISCUSSION

Growth

Sherman B diet. The literature on the effects of radiation upon the growth of organisms is conflicting. Rats have been shown to grow equally well in darkness as in well-lighted rooms when the diet is optimal (Goldblatt and Soames, '23). On the other hand, Ludwig and von Reis ('31) report increased growth of rats reared under visible red radiation. Brown ('28) studied light environment and its effect on rabbits and demonstrated a growth-promoting effect of exposure to neon light which was greater than in animals receiving sunlight through window glass, or those reared in darkness.

One hesitates, in the absence of more quantitative feeding trials, to lay too much stress on growth curves. There seems, however, little doubt that in our experiments an effect of visible radiation, and, to a lesser degree, of infra red radiation in promoting growth has been demonstrated.

This effect is most marked in the third generation. The effect appears during lactation: the birth weight in all groups is identical but the curves for 'light' and 'dark' rats diverge after the fifth day. The intermediate position between 'dark' and 'light' of the two groups kept in infra red radiation makes it difficult to rule out an effect of these radiations. These results could be interpreted to mean some failure of function in the anterior hypophysis in animals deprived of light, resulting in lessened production of the lactogenic hormone in the mother, or of growth hormone in the young, an effect which according to some authors may be due to lack of retinal stimulation by light (Bissonnette, '38). Our results, although suggestive, afford no proof of this.

'McCollum' diet. We have no means of knowing the amount of fluorine ingested by the rats on this diet. It was sufficient to produce a chronic fluorosis. There is abundant evidence in the literature that fluorine added to the normal diet of rats inhibits growth (Iowa Agricultural Experiment Station reports, '28, '29; Goldemburg, '27; McClure and Mitchell, '31).

The growth inhibitory effect of fluorine in rats deprived of light is clearly seen in our curves. It is interesting to note, however, that radiation, both visual and infra red, overcomes, to a marked extent, the growth-inhibiting effects of fluorine. We have already described a lack of pigment in the teeth, due to fluorine, which occurred earlier in the group of rats deprived of light (Hodge, Luce-Clausen and Brown, '39).

Vitamin A storage—both diets

If we admit the validity of the statistical methods applied to our data, and it seems justifiable to do so, we are surprised to find that the rate of vitamin A storage, with increased weight at given ages, is faster in the groups of rats deprived of light than in those exposed to visible radiation, though the trend of the slope for increased weight is upward in both groups. The presence of fluorine in the 'McCollum' diet seems only to magnify the effect observed in the groups fed the uncontaminated diet.

In considering the factors which might decrease the storage of vitamin A in the 'light room' as compared with the 'dark room' rats several conditions may have been operative.

(1) Some increased use of vitamin A for bodily functions such as restoration of tissues, growth, and cell metabolism in the 'light room' rats, the males especially.

(2) A faster disappearance of vitamin A from the skin of the irradiated rats.

(3) Heat. McCoord ('38) has shown that hyperthermia and fever cause the supply of vitamin A to disappear from the liver. The rats in room III, especially during the summer months, were, from time to time, exposed to higher temperatures than were those in room I. This may have produced an effect on activity and appetite but the amount of heat was not sufficient to raise rectal temperature.

(4) Retinal stimulation. Tansley ('31) studied the mechanism of dark adaptation in albino rats. Using an illumination of 400 foot candles she found that ½ hour's exposure was sufficient to cause complete bleaching of the retina.

The only illumination used in our dark room was that from a single 7.5 watt green Mazda lamp placed 5 feet away from the cages. This was turned on for a short time each day for routine procedures. No rat was ever exposed to it for a longer time than that required to clean a cage, to weigh, or, in the case of females, to take a vaginal smear.

Measurements taken with a Weston foot candle meter, model 614, showed zero f.c. at 10 inches and a maximum of 16 f.c. at a distance of 0.5 inches. It therefore seems safe to say that the retinas of our animals were never, at any time, bleached by light. In room III the illumination was 300 f.c. and rats were exposed to it for 12 hours daily.

The researches of Wald ('35) have shown that small amounts of vitamin A are used up in the retina, and that light adapted retinas contain about 0.2–0.3 micrograms of vitamin A; dark adapted ones, only a trace. The ability of the retina to form visual purple after exposure to light depends on the supply of vitamin A. The turnover of vitamin A in the retina depends on the exposure of the retina to light. It is reasonable to suppose that complete absence of retinal stimulation, in a group of rats deprived of light, might lead to some retention of vitamin A that, under normal conditions of retinal stimulation by light, would be used up in the retina.

No quantitative data as to the amount of vitamin A used in retinal stimulation by light are available. Neither do we know whether the constant circulation of vitamin A in the retina would utilize enough to deplete liver stores, or, in the absence of stimulation, to augment them. It may be suggestive that the difference of storage between animals in rooms I and III is small, and for this reason more readily explainable on this basis.

CONCLUSIONS

1. Radiation in the visible region of the spectrum promotes growth in rats fed the Sherman B diet, as compared with groups fed the same diet and kept in complete darkness. This effect is more evident in the third generation of rats bred under controlled conditions of light, and more marked in males than in females.

2. Radiations in the near and far infra red regions have a growth-promoting effect which is definite but lesser in degree than that of visible light.

3. Visible radiation, and, to a lesser degree, infra red radiation, compensate for the marked inhibition of growth due to fluorosis seen in rats deprived of light.

4. Rats kept in darkness and fed the Sherman B diet, and also groups fed a diet contaminated with fluorine are shown to store vitamin A in livers at a more rapid rate for weight at given ages than do comparable groups exposed for 12 hours a day to visible light. It is suggested that lack of retinal stimulation by light may play a part in this result.

In conclusion, our thanks are due to the following: The Eastman Kodak Company for continued interest, support, and collaboration; the General Electric Company and the Westinghouse Company for special cooperation in regard to the supply of lamps; and the Corning Glass Company for interest and kind cooperation.

We also wish to thank Dr. Donald R. Charles for suggesting the mathematical methods employed in the analysis of data and for much helpful criticism; and Dr. Nancy Morey and Miss Eugenia Sheridan for help in the routine care of animals.

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THE USE OF ISOLATED RADIATION IN EXPERIMENTS WITH THE RAT

III. EFFECTS OF DARKNESS, VISIBLE AND INFRA RED RADIATION ON THREE SUCCEEDING GENERATIONS OF RATS

(*b*) REPRODUCTION [1]

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SIX FIGURES

(Received for publication July 31, 1939)

INTRODUCTION

In an earlier paper ('39) we have described results on growth and storage of vitamin A on three succeeding generations of rats kept continuously in the following environments: (a) darkness, (b) visible radiation, (c) infra red radiation. A full description of the rooms used in these experiments has also been given (Jones and Tuttle, '39).

This paper deals with the results on reproduction in the same rats. The rooms are designated room I (dark), room II (near infra red), room III (visible), and room IV (far infra red).

EXPERIMENTAL PROCEDURES

The date of opening of the vagina was carefully noted in all female rats. Daily records of vaginal smears, taken according to the technique of Long and Evans ('22), were kept, to determine the number of oestrous cycles up to the date of mating. Eight females, four on each diet used, the Sherman B (Sherman and Campbell, '24) and McCollum (Evans and Bishop, '22), were selected as breeders, along with four males, two on

[1] Aided by a grant from the Committee on the Effects of Radiation upon Living Organisms, Divisions of Biology and Agriculture, National Research Council.

each diet, in each room. Oestrous records were kept on all remaining female rats. For smearing we used for each rat an individual glass spatula sterilized daily.

At the age of 110 days, each female in the pro-oestrous stage, was placed with a male. The presence of vaginal plug and of sperm were taken as evidence of positive mating. Smears were taken till the fourteenth day when the appearance of a clot gave evidence of implantation. Smearing was then discontinued until after the birth of young; the animals were weighed daily during the last week of pregnancy. The number of young born were counted, and each litter was weighed daily for the first 17 days.

Results on the two diets are given separately. As explained in the previous paper an accidental contamination with fluorine of the commercial casein used in the McCollum diet was discovered and described (Hodge, Luce-Clausen and Brown, '39). The designation 'McCollum' signifies the diet plus its contamination.

RESULTS

1. Age of opening of the vagina, oestrous cycles

The age of opening of the vagina in all female rats is given in frequency polygons, those for the rats fed the Sherman B diet being shown in figure 1, and those for the 'McCollum' diet in figure 2. Details of oestrous cycles of females fed the same two diets are given in figures 3, 4, 5.

The total number of observations in the three groups given in figure 1 is 105, in figure 2, 135. The results for rats in rooms II and IV were so nearly identical that, for the sake of clearness, they have been plotted together.

Table 1 gives a summary of data obtained on the age of opening of the vagina in the six groups. It shows that on both diets the age of opening of the vagina is even earlier than normal [2] in rats receiving visible radiation (room III), the

[2] Since these experiments were concluded we have studied the age of opening of the vagina in ninety-three rats, of the Sprague-Dawley strain, fed the Sherman B diet and kept under ordinary conditions of light in our laboratory. The average age for these rats is 45.9 ± 0.4 days—or about the same as was obtained in rooms II and IV. This indicates that the 42-day figure for rats in room III is earlier than might be expected for rats kept under our experimental conditions.

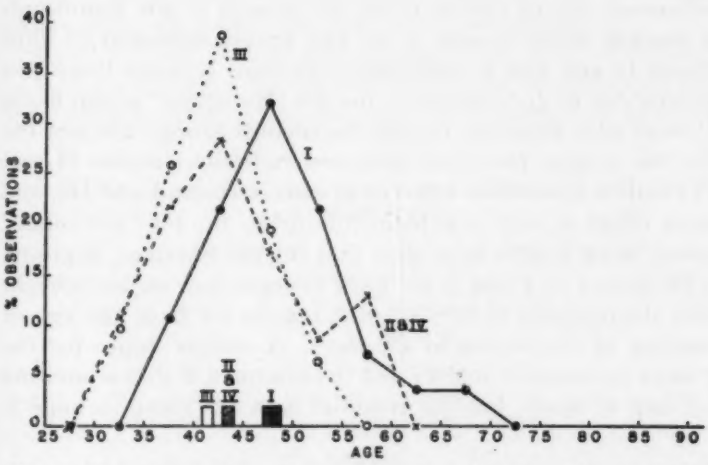

Fig. 1 Frequency polygons of age of opening of vagina. Sherman B diet. I = rats kept in complete darkness; II and IV = rats exposed to near and far infra red radiation; III = rats exposed to visible radiation.

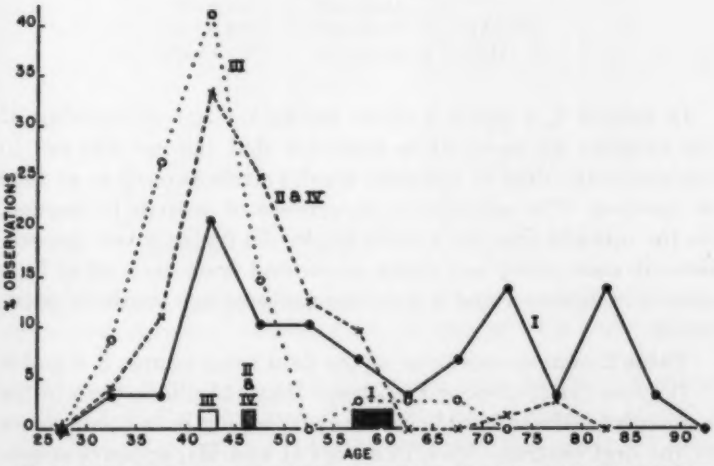

Fig. 2 Frequency polygons of age of opening of vagina. 'McCollum' diet. I = rats kept in complete darkness; II and IV = rats exposed to near and far infra red radiation; III = rats exposed to visible radiation.

difference due to diet in these two groups is not significant. A marked delay is seen in the two groups deprived of light (room I) and also a significant difference between these two groups due to diet, the mean for the 'McCollum' group being 11 days later than that for the Sherman B group. The age for the two groups receiving infra red radiation (rooms II and IV) falls intermediate between groups in rooms I and III, and some effect of diet is evident, the mean for the 'McCollum' group being 3 days later than that for the Sherman B group.

In figures 3, 4 and 5 we have represented, on an upright line, the oestrous history of each female rat from the age of opening of the vagina to 120 days. A similar figure for the groups in rooms II and IV fed the Sherman B diet is omitted for lack of space, but the essential data are given in table 2.

TABLE 1

Age of opening of the vagina

ROOM	SHERMAN B DIET	'MCCOLLUM' DIET
	days P.E.	*days P.E.*
I	48.3±0.92	59.3±2.06
II and IV	44.0±0.75	46.8±0.70
III	41.8±0.65	42.7±0.90

In figures 3, 4 and 5 a circle marks the age of opening of the vagina. An open circle indicates that the rat was not in oestrus at the time of opening, a solid circle in oestrus at time of opening. The subsequent occurrence of oestrus is marked, on the upright line, by a solid circle. In figure 3 two generations in each group are given, separated from each other by a space; in figures 4 and 5 three generations are similarly separated.

Table 2 summarizes some of the data from figures 3, 4 and 5. It gives fairly clear-cut evidence that radiations both in the near infra red and visible regions play a part in the occurrence of the first oestrus. This, in rooms II and III, appears coincident with the opening of the vagina in 80–90% of the animals, whereas the percentages for rooms I and IV are definitely

lower. The presence of fluorine in the diet of the 'McCollum' group does not seem to have affected this result.

Table 2 shows a mean lengthening of the cycle of 1.6 days in the rats in room I fed the Sherman B diet as compared with the group in room III on the same diet. According to the χ^2

TABLE 2

Occurrence of oestrus at time, or within 2 days, of opening of vagina and mean length of cycles

ROOM	SHERMAN B DIET				'MCCOLLUM' DIET			
	In oestrus at time of opening	Mean length of cycle	S.D.	Coefficient of variability	In oestrus at time of opening	Mean length of cycle	S.D.	Coefficient of variability
	%	*days*			%	*days*		
I	45.0	7.3	4.7	53.1	52.2	6.1	3.1	46.2
II	81.8	5.6	..	...	90.0	5.3	..	...
III	80.0	5.7	3.1	53.1	80.0	5.7	2.7	44.0
IV	64.3	6.5	..	...	56.0	6.5	..	...

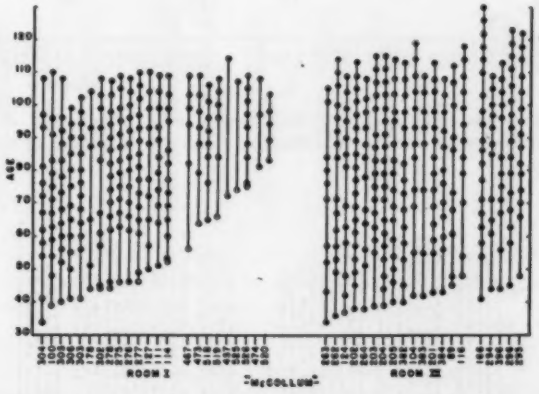

Fig. 3 Oestrous history of individual female rats. 'McCollum' diet. Room I—rats kept in complete darkness. Room III—rats exposed to visible radiation.

method of analysis this result gave a probability of 1 chance in 10,000 in the Sherman B group, and 40 chances in 100 in the 'McCollum' group that the longer cycles observed arose as a matter of chance. We can conclude therefore that in this set of observations the lengthening of the cycles in room I of

rats on the Sherman B diet was a definite finding. There was no significant lengthening of cycles in room I of rats on the 'McCollum' diet. The apparent discrepancy here is possibly explained by the better nutritional value of the 'McCollum' diet despite its contamination.

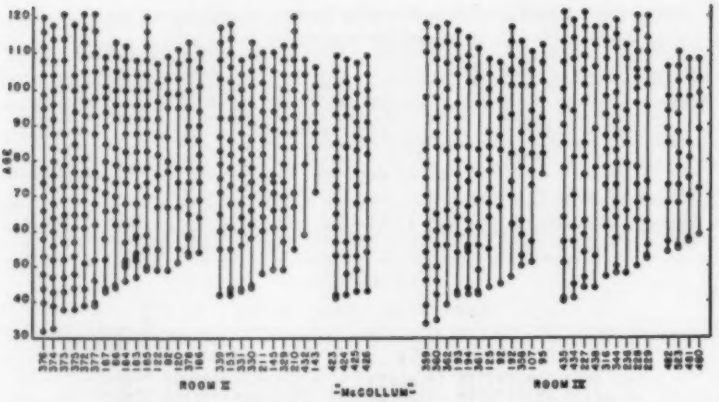

Fig. 4 Oestrous history of individual female rats. 'McCollum' diet. Room II—rats exposed to near infra red radiation. Room IV—rats exposed to far infra red radiaton.

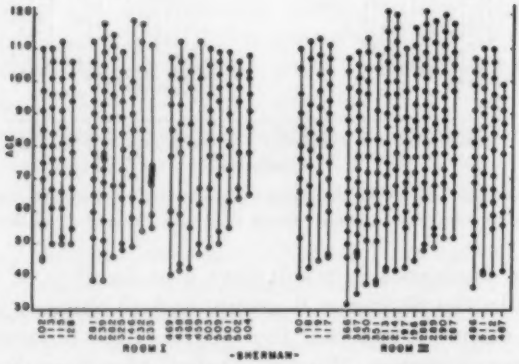

Fig. 5 Oestrous history of individual female rats. Sherman B diet. Room I—rats kept in complete darkness. Room III—rats exposed to visible radiation.

Owing however to the many factors which might produce variability in results, this observation would have to be confirmed. It is evident by inspection of figures 3, 4 and 5 that lengthening of the cycles, in individual rats, occurred at times in all the groups. It is also seen that more rats were affected in this way in room I, and more on the Sherman B than the 'McCollum' diet.

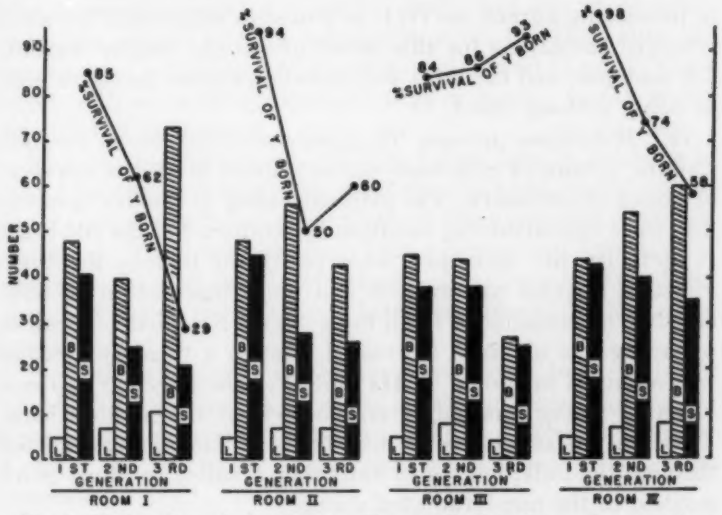

Fig. 6 Birth and survival of young. Sherman B diet. L—number of litters; B—number of young born; S—number of young that survived.

2. Birth and survival of young

(*a*) *Sherman B groups, three generations.* Results are given in figure 6 where the number of litters (L), the total number of young born (B) and the number survived (S) are represented for each generation in each room. The percentage survival of the number of young born in each generation is also indicated.

The percentage survival of young born is normal in all the groups in the first generation. These young were the offspring

of the original pregnant females sent to us from The Wistar Institute.

In room I the failure to survive falls from 62% to 29% in the second and third generations. In room III a normal survival is maintained through three generations. In rooms II and IV the survival in the second and third generations is not normal but superior to results obtained in room I.

It would seem from this that radiation plays a definite role in promoting normal survival of young in rats—that the most effective radiations for this effect are in the visible regions, but that near and far infra red radiation cannot be considered as being without effect.

(*b*) *'McCollum' groups.* The presence of fluorine in the 'McCollum' groups of rats made our results on birth and survival of young inconclusive. The original young of the first generation were reared during lactation by mothers fed the Sherman B diet. By the time they were ready for mating the toxic effect of fluorine was evident and there was only a 50–63% survival of young born in all the groups. No third generation of young was obtained in room I, though a third generation was obtained in rooms II, III and IV. The survival of these radiated young was only from 35–46% of the number born. These results suggest some toxic effect of fluorine transmitted through the milk, but, as in our other results, an effect more marked in the non-irradiated group.

DISCUSSION OF RESULTS

Effect of darkness versus radiation

(*a*) *On rats suffering from fluorosis.* The literature on this subject is scanty. Chaneles ('29) has stated that teeth deformities due to fluorosis in a group of rats which received radiation from a Hanau lamp were less marked than in a similar group which were not irradiated. Smith and Smith ('32) observed that in human teeth, the deposition of pigment, a secondary phenomenon due to fluorosis, occurs on the lip line, or the part of the tooth exposed to light.

It seems to us that the problem of the effect of light on fluorosis merits further study. It is significant, in our results, that the marked delay in the opening of the vagina seen in the second generation of 'McCollum' rats in room I was not apparent in a completely comparable group in room III (fig. 3). Furthermore this delay was not seen in thirty rats from room II, or in twenty-four from room IV (fig. 4), under identical dietary conditions. It is evident from the frequency polygons (fig. 2) that the one for room I includes two sets of observations, the second being a later generation, but that, since all generations are included in polygons III, and II and IV, an effect with succeeding generations is only seen in the group deprived of radiation.

In the teeth of these rats (Hodge, Luce-Clausen and Brown, '39) a lack of pigment in those deprived of light was observed at an earlier date than in the teeth of those exposed to visible radiation. The growth curves (Luce-Clausen and Brown, '39) showed a marked failure of growth of the rats kept in darkness which was not apparent in radiated rats fed the same contaminated diet.

It would seem that all these results point to a combined effect of darkness plus fluorosis on the animals deprived of light. Also that radiation compensates to a demonstrable extent for the full effect of fluorosis as seen in the dark-room animals.

A delay in the onset of maturity in female rats deprived of light appears to be a definite finding. A recent paper by Fiske ('39) suggests that the balance of the two gonadotropic hormones in rats is changed under varying conditions of light. Fiske also observed delay in the onset of maturity in rats kept in darkness. The prolonged periods of oestrus in rats kept under continuous exposure to light which she observed were not apparent in our animals kept under 12-hour alternate exposures to light and darkness. This agrees with the findings of Browman ('37).

We have some evidence that the cycles in female rats in room I were lengthened, and very definite evidence that with

succeeding generations the young born in this room did not survive normally. This failure to suryive in young born in the dark increased in the second over the first, and in the third over the second generations. It appeared to be due to some failure in lactation. The young born were normal in birth weight, appeared to be active and healthy, but many died before the fifth day. The mothers, in many instances, appeared nervous, did not make good nests, and showed no inclination to take care of the young. This result again suggests decreased pituitary activity with failure to produce the lactogenic hormone.

The powerful effect of pituitary hormones upon growth and reproductive processes is well known (Evans and Long, '21; Evans and Simpson, '29, '31). It is tempting, from some of our studies, to add radiation, operating through the anterior pituitary in the rat, to the complicated chain of reproductive events. We can only, however, regard this idea as an avenue of approach for future work.

Radiations responsible for the effects described. Radiations throughout the visible and infra red regions of the spectrum have been shown to be effective in promoting growth, early opening of the vagina, early onset of oestrus, and normal survival of young born, in normal animals.

Whatever the biological explanation of these results may be, we have to consider certain possibilities with regard to radiation. Are all the radiations in the spectrum equally effective, and, if so, cannot we regard the effect of radiant energy on the rat, in the spectral regions studied, as simply one of heat with possible resulting catalysis of many chemical reactions in the body? This, at first sight, would appear to be a likely explanation, and is supported by the identical results obtained in rooms II and IV. In these two rooms the rats were exposed to equal energy equivalents, 0.1 gm. cal. per cm.^{2} per min. (Jones and Tuttle, '39). In room III, however, the picture is different. If we discount secondary radiations which resulted from time to time from heat, the actual amount of energy in the visible was roughly one-third that obtained in rooms II and IV (0.03 gm. cal. per. cm.^{2} per min.). If we include the

secondary effect of heat, the total energy in room III still falls below that of rooms II and IV (0.08 gm. cal. per cm.^{2} per min.), so that with less energy we get more effect. These results therefore show a peak of maximum efficiency here which seems to be independent of total energy, and obviously referable to illumination. In room III the illumination was 300 foot candles (Jones and Tuttle, '39).

It was surprising to us that any effects at all could be demonstrated in room IV, where the only radiations present were long wave-length infra red. We should have expected results in this room to approximate those in room I, instead of which, in practically every case, they were similar to those in room II. For this reason an effect of radiation was apparent, and was probably only brought out by the efforts made to eliminate all known sources of radiation from room I, and to keep all four rooms at the same air temperature.

It would be very desirable to sub-divide the visible region further, and find out the region of maximum effect. At the time these experiments were planned there was no satisfactory way of doing this, since the light sources available did not emit radiations of sufficient intensity in certain regions, for example, the blue.

CONCLUSIONS

1. The age of opening of the vagina and the onset of first oestrus in female rats is delayed when rats are bred in complete darkness. Radiations in the visible region of the spectrum promote an age of opening of the vagina which is earlier than normal for rats kept under our experimental conditions. Radiations in the near and far infra red promote an age of opening of the vagina similar to that obtained under ordinary conditions of laboratory light here.

2. Radiation plays a definite part in promoting the normal survival of young born in succeeding generations of rats reared under controlled conditions of light. The most effective radiations are in the visible, but radiations in near and far infra red are not without effect.

3. Visible radiation, and, to a lesser extent, infra red radiation, compensate, to a demonstrable effect, for a delay in the

age of opening of the vagina, which increases with succeeding generations, in rats suffering from chronic fluorosis and deprived of light.

4. Radiation, throughout the visible and infra red spectrum, plays a part in reproductive processes in the rat. In the near and far infra red equal results have been demonstrated, using equal energy equivalents in these two regions. Using in the visible region one-third the energy of that used in the infra red, the best results were obtained.

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ADAPTATION TO A LOW CALCIUM INTAKE IN REFERENCE TO THE CALCIUM REQUIREMENTS OF A TROPICAL POPULATION

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ONE FIGURE

(Received for publication April 25, 1939)

Much has been written concerning the calcium requirements of children and adults. Most of the work which throws light upon this matter has been done in Europe and America, and the conclusions which have been drawn are such that they do not appear to be applicable to the conditions in many tropical countries. Sherman ('37) sets the standard allowance for maintenance of the adult at 0.68 gm. and 1 gm. for the growing child. He also states, "It is doubtless better for family food supplies to furnish at least 1 gm. per capita per day."

Leitch ('37) considered the calcium requirements for children and adolescents to range from 0.9 gm. between the ages of 5 and 9, to 1.9 gm. between the ages of 15 and 16 years, and thereafter to decrease gradually to 0.55 gm. at the age of 24 years.

The diets of many tropical races consist of cereals, pulses, roots, vegetables and vegetable oils; milk and eggs seldom appear. Our studies are concerned with Ceylon, where the majority of the poorer classes augment such diets with a little fish. A typical diet of the laboring classes with a value of about 3000 calories, which does not include sprats, contains 0.3 gm. calcium daily. But many of the laboring class Ceylonese take sprats two or three times a week, and this increases the amount of calcium to 0.6 gm. (Nicholls, '37).

The daily diet of the average adult Ceylonese has a value of about 2400 calories and children of 5 years of age consume diets of a value of about 900 calories and those of 10 years of age of about 1800 calories. The amount of calcium in the diets of the children of these ages is a little less than 0.2 and 0.4 gm. respectively. Children are weaned to these diets and the great majority of them never receive milk. The records of one survey of 4221 daily diets of young school children showed that only 251 had received milk (Nicholls, '36).

However deficient such quantities of calcium may appear, the fact that the majority of children become adults shows that they make shift to grow on these quantities. Hence, the question of individual and racial metabolic adaptation arises.

METABOLIC ADAPTATION TO LOW CALCIUM INTAKE

Urinary calcium

Assuming for the sake of argument that there is good absorption of calcium from the alimentary tract whether the total intake is much below or equal to optimum requirements, then if a metabolic adaptation is to take place, it must be in the amount excreted. Sixty-two samples of urine of boys of the laboring classes and an equal number from upper-class boys of the Royal College were analyzed for calcium content. The records of diets of the latter (Nicholls, '37) show that they receive about 1 gm. calcium daily. Table 1 gives the results.

The percentages of table 1 are presented in figure 1, which shows the marked contrast in the amount of urinary calcium in these two groups of boys of different social status.

Calcium balances in growing children

Calcium balances were carried out with four children of the laboring classes. They were given diets similar to those they had received in their homes. The diets were duplicated, one being reserved for calcium analysis. These determinations were made for a period of 3 days for three children, and of

TABLE 1

The calcium content of the urine of upper and lower class boys in Ceylon

AMOUNT OF CALCIUM IN 100 CC. OF URINE	ROYAL COLLEGE		LABORING CLASS	
gm.	*number*	%	*number*	%
0.0010–0.0030	7	11.3	36	58.1
0.0030–0.0050	11	17.7	19	30.6
0.0050–0.0070	13	21.0	1	1.6
0.0070–0.0090	9	14.5	0	0
0.0090–0.0110	5	8.1	3	4.8
0.0110–0.0130	3	4.8	1	1.6
0.0130–0.0150	3	4.8	0	...
0.0150–0.0170	3	4.8	0	...
0.0170–0.0190	3	4.8	0	...
0.0190 and above	5	8.2	2	3.3
Average amount of calcium in 100 cc. of urine (gm.)	0.0096		0.0041	

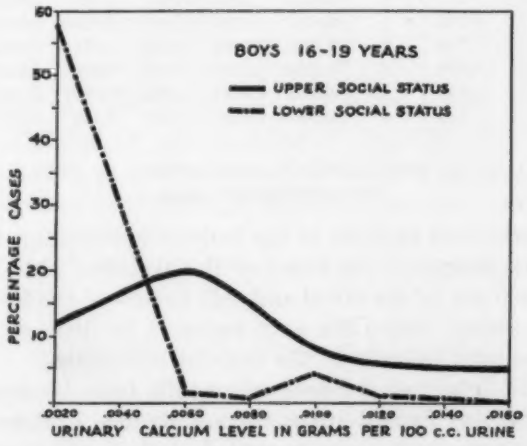

Figure 1

9 days for a fourth child (no. 2). All the children had positive calcium balances, and those on low calcium intakes of about 0.2 gm. a day were able to absorb and retain from 34% to 89% of the calcium (table 2). Child no. 2 for the first 3 days of the experiment had a total daily intake of 0.07 gm. of calcium and retained 0.05 gm. of it.

These children obtain all but a very small amount of vitamin D through the direct action of the sun's rays on their skins and these high retention rates indicate that they receive in this manner an ample quantity of vitamin D.

Table 2 gives the details of these experiments in calcium balances.

TABLE 2

Calcium balances in children

NO. OF CHILD	WEIGHT OF CHILD	AGE AND SEX	PERIOD	DAILY TOTAL INTAKE OF Ca	DAILY TOTAL URINARY EXCRETION OF Ca	DAILY TOTAL FAECAL EXCRETION OF Ca	TOTAL DAILY EXCRETION	DAILY RETENTION OF Ca	Ca RETAINED
	kg.		*days*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
1	16.6	7 F	3	0.2454	0.0287	0.1352	0.1639	0.0815	34
2	17.3	7 M	3	0.0703	0.0035	0.0154	0.0189	0.0514	73
2	17.3	7 M	3	0.2225	0.0066	0.0472	0.0538	0.1687	87
2	17.3	7 M	3	0.2196	0.0049	0.0452	0.0501	0.1695	89
3	10.9	4 M	3	0.2051	0.0251	0.0554	0.0805	0.1246	71
4	12.5	4 M	3	0.1833	0.0240	0.0960	0.120	0.0633	41

THE AMOUNT OF CALCIUM IN THE BODIES OF CEYLONESE AT DIFFERENT AGES

The amount of calcium in the body is approximately 25%[1] of the dry weight of the bones of the skeleton, plus 10 to 12 mg. per 100 gm. of the blood and soft tissues of the body. The latter is almost negligible as it amounts to little more than 1% of the total calcium of the skeletal structures.

Many skeletons of children and adults have been collected in the Anatomy School of the Ceylon Medical College. In all cases the ages have been recorded, and in most cases the heights and weights of the cadavers of the children are known.

[1] Bone ash having a composition of 6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$ is about 70% of the dried bone, which is approximately 25% calcium. Analysis of six samples of dried bone ranged from 22.8–24.9 with an average of 24% calcium.

Prof. W. C. O. Hill kindly allowed us to take the weights and measurements which we required of these skeletons; and in a few cases he permitted the removal of samples of bones for analysis.

Calcium in the skeletons of children

The soft tissues had been removed soon after death and the skeletons cleaned but the bones remained articulated with the ligaments, cartilages, intervertebral discs and epiphyses attached. Each skeleton was weighed and placed in an oven at 100°C. for 6 hours and the dry weight taken. The loss of weight varied from 9.6 to 11.8%. Ten per cent was deducted

TABLE 3

Estimated calcium in children

AGE AND SEX	RACE	BODY WEIGHT	DRY WEIGHT OF ARTICULATED SKELETON	ESTIMATED WEIGHT OF Ca IN SKELETON	
years		*kg.*	*gm.*	*gm.*	*gm./kg.*
3½ F	Sinhalese	13.20	640	144	10.9
4½ F	Sinhalese	14.12	680	155	10.9
5 M	Sinhalese	17.31	824	206	11.9
6 F	Tamil	16.15	770	192	11.8
8 F	Sinhalese	18.80	937	234	12.4
11 M	Sinhalese	23.20	1247	312	13.4

from the weight of each skeleton to allow for the ligaments, cartilages, etc., and the amount of calcium in each skeleton was calculated as 25% of the skeleton after this deduction.

Table 3 gives the details for six of these skeletons and the last column gives the weight of calcium in grams per kilogram body weight.

Calcium in the skeletons of adults

Fifteen skeletons from males and fourteen from females were weighed, and the femurs weighed and measured. Ten samples of bones with contained marrow were taken from these skeletons. They were dried for 3 hours at 105°C. and lost between 8.9 to 10.1% in weight, the average loss being 9.5%. The ash ranged from 64 to 68.4% of the dried samples.

The dried weight of the skeletons was computed by deducting 9.5% for moisture from the actual weight of the bones. The height in life of the person from which the skeleton came has been calculated from the length of the femur by using Pearson's formula. And from tables of the average weight for height for males and females of the various races in Ceylon at different ages, the probable weight of the person from which each skeleton came has been calculated. The average height of the males by these calculations was 164.5 cm. (5 feet, 4¾ inches). The average weight for this height is approximately 51 kg.; this gives the amount of calcium per kilogram body weight as 16.5 gm.

The average height of the females by these calculations was 151.6 cm. (4 feet, 11¾ inches). The average weight for this height is approximately 41 kg. This gives the amount of calcium per kilogram body weight as 15.2 gm.

The details of these computations are given in table 4 for males and table 5 for females.

Leitch ('37) has calculated the amount of calcium in the body as 30 gm. per kilogram at the age of 5 years, and as 36 gm. from the age of 12 years, 36 gm. per kilogram also being the figure for adults. These figures are more than double the averages obtained by the methods here described; and obviously the amounts of calcium required for growth at different ages, if calculated on the basis of the figures given here will be greatly at variance with the amounts calculated by her figures.

There are no complete European skeletons available in Ceylon for our purposes, but we obtained one femur of a European male and another femur from a European female. We found that the weight of the femur of the skeletons studied by us had a fairly constant relationship to the weight of the entire skeleton, the average being 8.7% for both males and females (column 5, tables 4 and 5). We calculated the entire weights of the skeletons of the Europeans from which the two femurs had come by using this figure. Our calculations are given in the last lines of tables 4 and 5.

TABLE 4

The relation of femur length to size and calcium content of the skeleton: males of India and Ceylon

AGE	RACE	WEIGHT OF SKELETON	WEIGHT OF FEMUR	WEIGHT OF FEMUR IN % OF SKELETON	LENGTH OF FEMUR	ESTIMATED HEIGHT (PEARSON)	ESTIMATED WEIGHT	Ca IN SKELETON	CALCIUM PER KILOGRAM BODY WEIGHT
years		*gm.*	*gm.*		*mm.*	*cm.*	*kg.*	*gm.*	*gm./kg.*
20	Sinhalese	3460	300	8.6	441	164.6	..	783	...
19	Sinhalese	3420	306	8.9	441	164.6	..	774	...
24	Sinhalese	4080	354	8.2	439	164.2	..	923	...
20	Sinhalese	3602	336	9.3	438	163.0	..	815	...
50	Sinhalese	4290	374	8.7	452	166.6	..	971	...
30	Malayalee	4090	350	8.2	448	166.0	..	925	...
50	Sinhalese	3870	337	8.7	439	164.2	..	876	...
30	Sinhalese	3740	304	8.1	436	162.3	..	845	...
21	Sinhalese	3927	349	8.8	443	165.0	..	890	...
30	Indian Tamil	3660	314	8.5	446	165.5	..	828	...
28	Indian Tamil	3900	345	8.8	443	165.0	..	883	...
50	Indian Tamil	3134	292	9.3	452	166.6	..	684	...
25	Indian Tamil	2723	241	8.8	426	161.7	..	616	...
40	Indian Tamil	3477	350	10.0	429	162.3	..	788	...
30	Ceylon Tamil	4482	382	8.4	452	166.6	..	1014	...
	Average	3724	329	8.8	442	164.5	51	841	16.5
	European	5295	452	..	460	167.8	65	1198	18.4

TABLE 5

The relation of femur length to size and calcium content of the skeleton: females of India and Ceylon

AGE	RACE	WEIGHT OF SKELETON	WEIGHT OF FEMUR	WEIGHT OF FEMUR IN % OF SKELETON	LENGTH OF FEMUR	ESTIMATED HEIGHT (PEARSON)	ESTIMATED BODY WEIGHT	Ca IN SKELETON	Ca PER KILOGRAM BODY WEIGHT
years		*gm.*	*gm.*		*mm.*	*cm.*	*kg.*	*gm.*	*gm.*
35	Sinhalese	3420	282	8.2	439	158.2	..	774	...
30	Sinhalese	3147	262	8.2	418	154.1	..	712	...
18	Sinhalese	2296	271	10.8	391	148.8	..	520	...
20	Sinhalese	2634	239	9.1	417	153.9	..	596	...
25	Sinhalese	2320	206	8.8	398	150.2	..	525	...
35	Sinhalese	2410	193	8.0	386	147.9	..	545	...
21	Sinhalese	2560	210	8.2	386	147.9	..	579	...
Aged	Sinhalese	2925	249	8.5	441	158.6	..	662	...
28	Sinhalese	3590	340	9.4	413	153.1	..	817	...
Aged	Tamil	2060	187	7.9	423	155.1	..	466	...
30	Tamil	2340	188	8.0	368	144.4	..	530	...
28	Tamil	2880	267	9.2	393	149.2	..	652	...
Aged	Tamil	2410	212	8.7	415	153.5	..	542	...
45	Tamil	3750	335	8.7	433	157.0	..	848	...
	Average	2767	246	8.7	409	151.6	41	626	15.2
	European	2850	248	..	437	157.8	48	645	13.4

The skeleton showed that the European woman was of light build, and we have guessed her weight to have been about 7½ stone (48 kg.). The skeleton of the male was of robust build, and 10¼ stone (65 kg.) is probably a conservative estimate. Although the method of estimating the amount of calcium in these European skeletons cannot be considered very satisfactory, yet it is sufficient to indicate that the amount of calcium per kilogram body weight is not markedly different in Ceylonese and Europeans.

Increase in the calcium in the body during the years of growth

The ultimate aim is to determine the calcium requirements for use in the prescription of diets; and as there are individual variations, within small limits, of the amount of calcium per kilogram body weight only round figures may be used, and these should be a little higher than the estimated average. The increase in the calcium in the body during the years of growth can be approximately determined by using the figures given in tables 3, 4 and 5 of the amount of calcium per kilogram body weight of children and adults. We base the requirements on 12 gm. of calcium per kilogram body weight for a child aged 5, and neglecting variations in growth, and allowing an increase of 0.5 gm. a year the figure 17 gm. per kilogram is reached at the age of 15 years.

The weights at different ages of children and adolescents of the various social classes have been published (Nicholls, '36).

The figures for males of the upper social status are for the youths and boys of the Royal College and its preparatory school; the figures for males of the lower social status are for boys of the free schools and others of the laboring classes. The former are considerably heavier at all ages. Table 6 gives the average weights of these boys at different ages, and from the amount of calcium per kilogram of body weight, the total amount of calcium in the body is calculated. From the increased amount of calcium in the body each year, the average daily retention throughout the previous year has been determined. The columns headed B.M. in table 6 are the weights

TABLE 6

Increase in the calcium in the body of males between the ages of 5 and 17 years

AGE	BODY WEIGHT			CALCIUM PER KILOGRAM BODY WEIGHT	CALCIUM IN BODY			INCREASE OF CALCIUM IN BODY SINCE PREVIOUS YEAR			DAILY RETENTION OF CALCIUM			CALCIUM REQUIREMENT:[4] U.S.S.
	L.S.S.[1]	U.S.S.[2]	B.M.[3]		L.S.S.	U.S.S.	B.M.	L.S.S.	U.S.S.	B.M.	L.S.S.	U.S.S.	B.M.	
years	*kg.*	*kg.*	*kg.*	*gm./kg.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
5	15.0	17.0	18.1	12	180	204	217	..	..	..	....	....	....	...
6	16.2	18.8	20.2	12.5	202	236	253	22	32	36	0.060	0.087	0.101	0.35
7	17.3	20.4	22.6	13	225	265	294	23	29	41	0.063	0.079	0.112	0.35
8	19.0	23.9	25.0	13.5	257	323	338	32	58	44	0.087	0.161	0.120	0.56
9	20.2	25.2	27.4	14	283	353	384	26	30	46	0.071	0.082	0.126	0.41
10	22.3	27.9	30.6	14.5	323	395	437	40	42	53	0.109	0.115	0.145	0.51
11	23.1	29.8	32.6	15	346	447	489	23	52	52	0.063	0.142	0.142	0.68
12	25.4	33.6	34.8	15.5	394	515	539	48	68	50	0.131	0.186	0.137	0.70
13	26.8	36.4	37.4	16	429	582	598	35	59	59	0.095	0.161	0.161	0.68
14	30.5	44.5	41.7	16.5	503	734	688	74	152	90	0.202	0.416	0.244	1.30
15	34.9	47.8	46.6	17	593	813	792	90	79	104	0.244	0.216	0.285	0.91
16	38.1	50.8	54.0	17	648	844	918	55	31	126	0.153	0.084	0.345	0.66
17	43.6	54.7	59.4	17	741	930	1010	93	86	92	0.254	0.238	0.249	1.10

[1] L.S.S.—males of lower social status.

[2] U.S.S.—males of upper social status.

[3] B.M.—British males.

[4] Calculated in grams as 2 × retention + 10 mg./kg.

and similar calculations for British males on the assumption that the average amount of calcium per kilogram body weight is more or less the same for any particular age group of males of any country.

Table 7 gives the figures for females. The two social statuses in this case are the girls of the primary and secondary schools. It was not possible to obtain the weights of girls of the highest social status.

It will be seen from table 6 that the daily retention of calcium by the L.S.S. males ranges from 0.06 gm. between the ages of 5 and 6 years to 0.254 gm. between the ages of 15 and 16 years. In the case of the U.S.S. males this retention ranges from 0.079 gm. between the ages of 6 and 7 years to 0.416 gm. between the ages of 13 and 14 years. The retention for British males is from 0.101 gm. between the ages of 5 and 6 years to 0.345 gm. between the ages of 15 and 16 years.

Table 7 shows that the retention rates are somewhat lower for females.

Leitch suggests that the daily calcium requirements should be double the daily retention rate plus 10 mg. per kilogram body weight. These suggested requirements are given in the last column of tables 6 and 7 and are calculated for the U.S.S. boys and the S.S. girls.

DISCUSSION

There are two factors among many which must be considered in relation to the question of calcium requirements. Firstly, growth does not take place at a uniform rate; the same applies to many, if not all metabolic processes. Periods of relatively great activity are succeeded by periods of less activity. This is shown for growth in any tables of the heights and weights of children at various ages. The Ceylon tables, from which the weights in tables 6 and 7 have been produced were drawn up from the measurements and weights of over 12,000 Ceylonese children; they show that the primary school girls have a rapid rate of growth between the ages of 7 and 8 followed by a very slow rate between the ages of 8 to 9; a

TABLE 7

Increase in the calcium in the body of females between the ages of 5 and 17 years

AGE	BODY WEIGHT			Ca PER KILOGRAM BODY WEIGHT	CALCIUM IN BODY			INCREASE OF CALCIUM IN BODY FROM PREVIOUS YEAR			DAILY RETENTION OF CALCIUM			CALCIUM REQUIREMENT:[4] S.S.G.
	P.S.G.[1]	S.S.G.[2]	B.G.[3]		P.S.G.	S.S.G.	B.G.	P.S.G.	S.S.G.	B.G.	P.S.G.	S.S.G.	B.G.	
years	*kg.*	*kg.*	*kg.*	*gm./kg.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
5	15.0	16.6	17.7	12	180	199	212	..	..	..	....	....	....	....
6	15.7	17.5	19.0	12.5	196	219	237	16	20	16	0.044	0.055	0.044	0.285
7	17.0	19.0	21.5	13	221	247	274	25	28	37	0.068	0.076	0.101	0.342
8	19.0	20.3	23.6	13.5	256	274	319	35	27	45	0.096	0.074	0.123	0.351
9	20.6	22.1	25.2	14	288	308	353	32	34	34	0.087	0.092	0.092	0.405
10	22.5	24.3	28.1	14.5	326	352	407	38	44	54	0.104	0.121	0.148	0.485
11	23.9	27.9	30.8	15	359	419	462	33	67	55	0.090	0.184	0.151	0.647
12	26.5	30.2	34.7	15.5	411	468	538	52	49	76	0.142	0.134	0.208	0.570
13	29.4	34.7	39.5	16	470	555	632	59	87	94	0.162	0.238	0.258	0.823
14	32.0	37.6	43.9	16	512	602	702	42	47	70	0.115	0.129	0.191	0.634
15	36.9	40.5	48.2	16	590	648	771	78	46	69	0.213	0.126	0.189	0.657
16	38.2	42.3	51.3	16	611	677	821	21	29	50	0.057	0.078	0.137	0.579
17	39.2	43.3	52.4	16	627	693	838	16	16	17	0.043	0.043	0.046	0.519

[1] P.S.G.—primary school girls.

[2] S.S.G.—secondary school girls.

[3] B.G.—British girls.

[4] Calculated in grams as 2 × retention + 10 mg./kg.

similar state of affairs occurs between 9 to 10 and 10 to 11. The secondary school girls are a year ahead, i.e., the years of rapid growth are 8 to 9 and 10 to 11, with both periods being followed by a slowing down of growth; similar pulsations of growth are shown in the tables for the boys. Even in tables drawn up from the statistics of very large numbers of children similar pulsations can be seen, although it might be expected that when dealing with large numbers the irregularities in the growth curve would be smoothed out, because some children are backward and others forward in growth.

It appears that when growth has been affected by ill health, or by poor diets, compensations take place. For instance, rapid rates of growth of the higher class Ceylon boys cease after the age of 17 years, but the poorest class boys have periods of rapid growth between the ages of 17 and 18 years; similarly the secondary school girls have no periods of rapid growth after the age of 15 years, whereas the free school girls have periods of moderately rapid growth after this age. But there is no complete compensation, because the averages for the poorer classes are in all cases far below the averages for the better classes.

The amount of calcium retained by growing children must be of the same order as the rate of growth. Not only are there long periods of high retention of a year or more, followed by long periods of low retention, but there are lesser pulsations in calcium retention extending over a number of days. The percentages of retention of the calcium intake, as shown in table 2 varied from 34% to 89%. Other observers have found great variations in the amount of calcium retained. Lusk ('28) gives an example of two children for whom calcium balances were determined for 10 days; K aged 5½ years had a daily intake of 0.91 gm. of calcium and retained 0.039 or about 4% of the intake; the second child N aged 7¾ had a daily intake of 1.3 gm. and the daily retention was 0.478[2] or about 36% of the intake. The child K was in a period of low calcium intake

[2] The figures of Lusk's table on page 573 are given in terms of CaO, and have been reduced to equivalents for Ca.

which could not continue or the child would become a dwarf, nor could the high calcium intake of the second child continue for long because the average daily amount of calcium required between the ages of 8 and 20 to produce a well-grown skeleton is less than 0.2 gm.

In the adult, calcium is continually being withdrawn from and returned to the skeleton, hence negative balances followed by positive balances must be a normal state of calcium metabolism.

The second factor is adaptation, whether individual or racial. If negative balances exceed the positive balances over a long period of time in any individual, osteoporosis must eventually supervene. Many races more or less vegetarian in food habits, especially in lands with long dry seasons, must make shift on a low calcium intake. In the dry zones of Ceylon leafy vegetables are not obtainable for many months, and as the inhabitants do not take milk or eggs, the diets are very low in calcium; yet there is no evidence of the existence of osteoporosis such as a high incidence of fractures, and the skeletons we have examined appear normal.

Sherman based his estimate of adult calcium requirement on metabolism experiments. When in any individual the intake was followed by retention he considered the requirements of the individual to be the amount of the intake less the amount retained; and when there was a negative balance the requirement was considered to be the amount of the intake plus the difference between the intake and the amount excreted—that is, the loss. Leitch also calculated the calcium requirements on calcium balance experiments. Neither of these authors takes into account the question of adaptations to various intakes. If a person is accustomed to a calcium intake of 0.6 gm. daily the metabolic processes will become adapted to this amount and the negative and positive balances will be more or less equal; but if suddenly his intake is reduced to 0.3 gm. his metabolism will not be adapted to this amount for sometime and consequently this intake will be followed by negative balances. On the other hand, if he has been accustomed to an

intake of only 0.3 gm., on an intake of this amount the negative and positive balances will be more or less equal.

It follows from this argument that, provided the calcium balances of an individual are determined over a sufficiently long period of time, all that such experiments will reveal is the daily amount of calcium the individual is accustomed to take; and calculation from these of optimum requirements cannot be made.

The heights and weights of the Ceylon children of the poorer classes compare unfavorably with those of the children of the upper, and it may be concluded that they are stunted. It may be that calcium deficiency plays a small part, but there is no clinical evidence of this; whereas the majority of the children of the poorer classes show marked signs of vitamin deficiencies, such as phrynoderma, sore mouth, Bitot's spots, xerophthalmia and night blindness at one time or another during the period of their growth, these signs are absent from the children of the upper classes (Nicholls, '34, '35, '36).

Therefore standard allowances may safely be based upon the requirements of the children of the upper classes, because they are well grown. If it is assumed that the amount of calcium per kilogram body weight is more or less the same in British children as in Ceylon children then these allowances should be applicable in Britain.

The calcium requirements calculated by the formula—grams of calcium retained $\times$ 2 + 10 mg. per kilogram body weight —give generous allowances. And the following, in a convenient form for practical use, are the standard allowances as calculated for the Ceylon children of the upper social status as given in the last columns of tables 6 and 7:

For children from age of 1 to 7 years—0.5 gm. of Ca.
For children from age of 7 to 12 years—0.75 gm. of Ca.
For adolescents from age 12 to 20 years—1.00 gm. of Ca.

A sufficiency of any constituent in the diets of healthy individuals is the optimum, nothing is gained by an excess, and as a general principle, dietary excesses should be avoided.

Whether or not a high intake of calcium over a long period is detrimental to health is unknown.

A standard allowance of 0.5 gm. of calcium daily appears to be sufficient for adults, excluding lactating women.

SUMMARY

1. The standard allowances for the daily intake of calcium for children and adults as given by authorities in Europe and America are considered too high.

2. There is evidence of metabolic adaptation to a low calcium intake as shown by: (a) The low amounts of calcium in the urines of those on a low calcium intake, when compared with the amounts in the urines of those on a high calcium intake. (b) The high percentage of calcium retention by growing children on about 0.2 gm. of calcium daily.

3. Many skeletons of Ceylonese children and adults have been weighed, and ten of these have been analyzed for moisture and ash. The amounts of calcium in the skeleton at different ages, and the amounts of calcium per kilogram body weight have been calculated.

The amounts of calcium retained daily by children and adolescents of different ages have been calculated from this.

4. Daily standard allowances for children and adults are suggested.

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THE ROLE OF THE ANTIDERMATOSIS VITAMIN AND A NEW WATER-SOLUBLE GROWTH FACTOR IN THE NUTRITION OF THE MATURE FOWL

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(Received for publication July 31, 1939)

Ringrose, Norris and Heuser ('31) were the first investigators to report the occurrence of dermatosis in chicks. Later Kline, Keenan, Elvehjem and Hart ('32) found that dermatosis, similar to that described by Ringrose and Norris but a more aggravated form, developed in chicks fed a diet that had been heated in a dry atmosphere for 144 hours at 100°C. Both groups of investigators considered that the factor preventing the dermatosis was vitamin G or B_2. Ringrose and Norris ('34) reported that the factor which prevented dermatosis in chicks was a complex consisting of a growth-promoting component, stable to prolonged dry heat treatment, and a dermatosis-preventing component, unstable under these conditions. Lepkovsky and Jukes ('35) also presented evidence that vitamin G consisted of two components, one of which was adsorbed upon fuller's earth while the other remained in the filtrate. Ringrose and Norris ('36 b) confirmed the results of Lepkovsky and Jukes showing that these components of the vitamin G complex are differentially adsorbed upon fuller's earth. The component remaining in the filtrate prevented dermatosis in chicks. It was called the filtrate factor by Lepkovsky and Jukes. These investigators found that the dermatosis-preventing factor was present in the filtrate obtained by treating a water-extract of rice bran with fuller's earth as well as in certain other materials.

Since the discovery of Kline and associates ('32) that the antidermatosis vitamin is destroyed by prolonged dry heat treatment, dermatosis has been frequently produced in the experimental laboratory. No evidence, however, that the mature fowl suffers from a deficiency of this vitamin has yet been reported. Hence, a study of this problem was undertaken, the results of which are presented in this paper. In the course of the study evidence was obtained that the experimental diets were deficient in another factor required for growth and reproduction as well as the antidermatosis vitamin. The results demonstrating this are also presented herein. Since the initiation of the study, Lepkovsky, Taylor, Jukes and Almquist ('38) have reported that the antidermatosis vitamin, under the conditions studied, seems to exercise no function in maintaining normal egg production and hatchability.

EXPERIMENTAL PROCEDURE

White Leghorn chicks and hens were used as experimental subjects in this study. The chicks were the progeny of hens fed in an adequate manner except where otherwise stated. All hens were reared and fed normally and were in full production at the time the experimental work was begun. All chicks and hens were selected for desired qualities at the beginning of each experiment after which they were distributed into groups. The pens in which the chicks and hens were confined were equipped with wire-mesh floors in order to reduce coprophagy to a minimum. In the work on reproduction the males were rotated among the lots frequently in order to rule out differences in breeding capacity. Both chicks and hens were identified by numbered wing or leg bands. The chicks were weighed weekly and the hens by periods of 2 weeks. The significance of the growth differences between the lots of chicks was calculated according to the method of Titus and Hammond ('35). Observations were made at time of weighing for the occurrence and severity of dermatosis in each chick. The degree of dermatosis per lot was then calculated according to the procedure of Ringrose and Norris ('36 a).

The diets used in this study were adapted after the heated diet used by Kline and associates ('32) in producing dermatosis in chicks. In order to render the diets free of the antidermatosis vitamin, the cereal portion and the liver extract (pre-mix) were heated in an oven for 36 hours at 120°C., and the casein was purified by the method of Sharp and DeTomasi.[1] Henceforth, the diets containing the unheated cereals are referred to as diet CA (chick adequate diet) and diet HA (hen adequate diet) and the diets free of the antidermatosis vitamin as diet CD (chick deficient diet) and diet HD (hen deficient diet). Table 1 gives the composition of diets CA and HA.

TABLE 1

Experimental diets

PRE-MIX	PARTS
Yellow corn meal	40.65
Wheat bran	20.00
Wheat flour middlings	20.00
Liver extract	0.35

	CHICK DIET %	HEN DIET %
Pre-mix	79.25	81.00
Purified casein	13.00	7.00
Crude expeller soy bean oil	4.00	5.00
Wheat germ oil	....	0.50
Cod liver oil	0.25	0.50
Pulverized limestone	2.00	3.50
Steamed bone meal	1.00	2.00
Iodized salt	0.50	0.50
Manganese carbonate	....	trace
Protein content	21.00	16.50

The cod liver oil used in these diets was a biologically assayed one, containing 3000 U.S.P. units of vitamin A per gram and 250 A.O.A.C. units of vitamin D per gram. According to Norris ('39), this oil in the amounts used provided quantities of vitamin A and D in excess of the requirements for growth and reproduction. Although vitamin E is stable

[1] Unpublished method by Sharp and DeTomasi of the Department of Dairy Husbandry, Cornell University.

to dry heat treatment, biologically assayed wheat germ oil was added to the hen diets in an amount that provided approximately ½ cc. of oil per hen per day. According to Card, Mitchell and Hamilton ('30), this quantity of wheat germ oil is sufficient to provide for the vitamin E needs of the hens exclusive of that in the cereal portion of the diet. The soy bean oil used in the diets was biologically assayed for vitamin K, using a modification of the method of Almquist and Stokstad ('37). In chicks fed diet CD a level of 4% promoted normal blood-clotting time. This oil was found to prevent chicks from developing nutritional encephalomalacia when fed the diet of Pappenheimer and Goettsch ('31); also it prevented the development of ataxic symptoms in chicks fed the vitamin B_4-deficient diet of Kline, Bird, Elvehjem and Hart ('36). The cod liver oil and wheat germ oil were added to the diets at intervals not greater than 7 days while the soy bean oil was added at the beginning of each experiment.

The deficient diets were found to be adequate in thiamin since the feeding of 75 μg. of thiamin hydrochloride per chick daily did not increase the growth of chicks fed diet CD above that of chicks receiving diet CD alone. Furthermore, polyneuritis was never observed in chicks fed this diet. These results therefore confirm those of Keenan, Kline, Elvehjem and Hart ('35).

Although no evidence has appeared showing that vitamin B_6 is essential in poultry nutrition, the deficient diets were proved to be adequate in this vitamin by means of the bioassay procedure of Bender and Supplee ('37), in which rat growth and the development of acrodynia are used as criteria. Nicotinic acid supplied either orally or parenterally failed to stimulate the growth of chicks fed diet CD above that obtained on diet CD alone. It was also ineffective in preventing or curing the dermatosis which develops in chicks fed this diet in agreement with results previously reported by Mickelsen, Waisman and Elvehjem ('38).

The results of fluorometric determinations by the method of Hodson and Norris ('38) showed that the liver extract here

used contained approximately 800 μg. of riboflavin per gram. This was in agreement with the results of a bioassay with chicks which showed that the liver extract contained approximately 770 μg. of riboflavin per gram. In this same bioassay it was found that the deficient diets contained 330 μg. of riboflavin per 100 gm., an amount in excess of that reported by Norris, Wilgus, Heiman, Ringrose and Heuser ('36) to be necessary for growth and reproduction. It was further found that the riboflavin content of the egg albumen of the eggs of hens fed diet HA and that of the eggs of the hens fed diet HD was approximately the same.

From the results of the bioassays of the deficient diets as well as those of certain of the special ingredients, it is evident that they were adequate in vitamins A, B, B_4, B_6, D, E, G, K, and the antiencephalomalacia factor. The diets were also either adequate in nicotinic acid or this vitamin is not required in poultry nutrition. These conclusions are supported by the failure of any characteristic symptoms of a deficiency of any of these vitamins to develop in the chicks and hens used in subsequent experiments.

Rice bran filtrate [2] prepared according to the method of Lepkovsky and Jukes ('35) was used as the source of the antidermatosis vitamin in this study. It was made from an aqueous extract of rice bran by twice subjecting the extract to fuller's earth adsorption, filtering off the fuller's earth, and concentrating the filtrate under vacuum. The filtrate, when assayed according to the method of Lepkovsky and Jukes ('36), was found to contain 25 units of the antidermatosis vitamin per gram.

Before beginning the work with hens it was felt desirable to determine whether or not diet CD, when supplemented with a sufficient quantity of the rice bran filtrate, would promote the same rate of growth as diet CA and thus be shown to be complete in all factors present in the latter diet, except the antidermatosis vitamin. Graded quantities of the rice bran filtrate were added to diet CD and fed to a corresponding

[2] Obtained from Vitab Products, Inc., Emeryville, California.

number of lots of chicks. One lot fed diet CD alone served as a negative control, and another fed diet CA as a positive control. The chicks were depleted of their reserves of the antidermatosis vitamin for 1 week before being placed upon experiment. The outline of the experiment and a summary of the results are presented in table 2.

Some dermatosis developed in nearly all lots during the first week that the chicks were on experiment as a consequence of the depletion during the previous week. In the negative control lot and the lots receiving 1 and 2% of the rice bran fil-

TABLE 2

Adequacy of diet CD when supplemented with rice bran filtrate (fourteen chicks per lot)

TREATMENT	DEGREE OF DERMATOSIS		MORTALITY AT 5 WEEKS	WEIGHT AT 5 WEEKS		SIGNIFICANCE OF DIFFERENCE
	At 2 weeks	At 5 weeks				
	%	%	%	gm.		't' [1]
Diet CD	6.4	37.6	7.2	89.0	>	2.29
CD + 1% rice bran filtrate	6.4	27.4	7.2	114.2	>	3.61
CD + 2% rice bran filtrate	5.5	19.7	7.2	152.1	>	2.25
CD + 4% rice bran filtrate	2.4	2.8	14.4	184.6	>	2.00
CD + 8% rice bran filtrate	1.6	0.0	7.2	206.0	>	5.44
Diet CA	0.0	0.0	7.2	266.5		

[1] t = 1.99, odds = 19: 1; t = 2.64, odds = 99: 1.

trate, the percentage degree of dermatosis gradually increased during the course of the experiment. In the lot receiving 4% of the filtrate, there was no appreciable increase in the degree of dermatosis, while in the lot receiving 8% the degree of dermatosis decreased. In a previous experiment the degree of dermatosis decreased slightly under identical circumstances in the lot receiving 4% of the filtrate. It is evident from these results that 4% of the filtrate supplied a sufficient amount of the antidermatosis vitamin to prevent the development of dermatosis. According to Almquist, Jukes and Newlon ('38) a chick diet containing 90 units of the antidermatosis vitamin per 100 gm. contains a sufficient amount for normal growth.

The growth of the chicks fed diet CD containing 8% of the rice bran filtrate was much less, however, than that attained on diet CA. The difference in growth was strikingly significant. On the other hand, the difference in growth between the lot fed the 8% level of the filtrate and the 4% level was barely significant. From these results it is evident that there is a growth factor lacking in diet CD which is not supplied in adequate amounts by rice bran filtrate and hence is not identical with the antidermatosis vitamin. Since diet CD was shown to be adequately supplied with all the known vitamins except the antidermatosis vitamin, the missing factor cannot be one of them. It is organic in nature, since it is destroyed by dry heat treatment. A preliminary report of these results has been made by Bauernfeind, Schumacher, Hodson, Norris and Heuser ('38).

In an attempt to find sources of the missing factor which could be used to make the deficient diets complete, when supplemented with the antidermatosis vitamin, resort was made to dried liver and liver extract, since Ringrose and Norris ('36 b) showed that a chick diet similar to diet CD when supplemented with dried liver promoted a superior rate of growth in chicks. Whey adsorbate was also used in this experiment, since from information available at this laboratory, it was possible to show that the growth response which Jukes ('37) obtained from this product could not be accounted for by its riboflavin content. The results of this experiment are presented in table 3. They show that these products at the levels used promoted growth which was significantly greater than that obtained in chicks fed diet CD supplemented with rice bran filtrate alone. The growth stimulated by these products was nearly as great as that promoted by diet CA.

In further work it was found that rice bran extract, rice bran adsorbate, dried brewer's yeast, autoclaved brewer's yeast, aqueous extract of brewer's yeast and grass juice promoted an increase in weight above that of the control lot which was strikingly significant in all but two instances. In these instances the growth difference was considerably better than

that required for mere statistical significance. The growth obtained in most instances was approximately equal to that obtained on diet CA.

These results showed that the factor lacking in the deficient diets, when supplemented with the antidermatosis vitamin, is water-soluble, adsorbed on fuller's earth and not destroyed in

TABLE 3

Sources of the new growth factor

(first experiment sixteen chicks per lot; second experiment twelve chicks per lot)

TREATMENT	MORTALITY	WEIGHT AT 5 WEEKS	SIGNIFICANCE OF DIFFERENCE
	Experiment 1		
	%	gm.	't' [1]
Diet CD + 6% rice bran filtrate (BD)	12.5	203.2	
BD + 300 μg. riboflavin per 100 gm.	6.3	208.3	0.4
BD + 4% whey adsorbate no. 1	5.3	235.0	2.5
BD + 3% liver adsorbate	0.0	239.1	2.8
BD + 0.35% liver extract	12.5	240.4	2.9
Diet CA	6.3	250.4	3.7
	Experiment 2		
			't' [2]
Diet CD + 6% rice bran filtrate (BD)	0.0	204.3	
BD + 300 μg. riboflavin per 100 gm.	0.0	208.8	0.4
Diet CD + 6% rice bran extract	0.0	254.1	3.7
BD + 4% rice bran adsorbate	8.3	243.8	2.9
BD + 1% whey adsorbate no. 2	0.0	246.3	3.0
BD + 5% brewer's yeast	0.0	308.5	7.7
BD + 5% autoclaved yeast	0.0	301.2	7.3
BD + aqueous extract of brewer's yeast ≏ 5%	8.3	276.3	4.9
BD + 25% grass juice	16.6	267.8	4.8
Diet CA	0.0	275.1	5.2

[1] First experiment t = 1.9, odds 19: 1; t = 2.6, odds 99: 1.

[2] Second experiment t = 2.0, odds 19: 1; t = 3.6, odds 99: 1.

yeast by autoclaving for 5 hours at 15-pound pressure. By treatment of rice bran extract with fuller's earth the new factor is separated from the antidermatosis vitamin and by prolonged dry heat treatment from riboflavin.

In the experimental work on the role of the antidermatosis vitamin in the nutrition of the mature fowl two experiments

were conducted. In both experiments the hens were fed diet HD alone until the hatchability of their eggs approached zero. The plan of both experiments and the results obtained are presented in table 4. In the first experiment the average hatch of the fertile eggs of the hens fed diet HD varied from 2.5 to 4.5% as compared to 60.8 to 68.8% for the eggs of the hens fed diet HA. When diet HD was supplemented with 5% of rice bran filtrate, a slight improvement in reproduction was

TABLE 4

Effect of the antidermatosis vitamin and the new growth factor upon the nutrition of the mature fowl

TREATMENT AND EXPERIMENTAL PERIOD	HENS PER PEN	LENGTH EXPERIMENTAL PERIOD	AVERAGE WEIGHT	AVERAGE EGGS PER HEN PER WEEK	EGGS SET	HATCH
		Experiment 1				
	no.	*weeks*	*gm.*	no.	no.	%
Diet HA (1)	24	8	1893	3.34	581	68.8
Diet HA (2)	23	6	1934	2.98	351	68.6
Diet HA (3)	21	6	1843	2.57	232	60.8
Diet HD (1)	14	8	1780	3.41	357	4.5
Diet HD (2)	13	6	1696	2.14	145	2.5
Diet HD (3)	6	6	1697	3.73	122	2.9
HD + 5% rice bran filtrate (1)	15	8	1772	3.50	416	12.3
HD + 10% rice bran filtrate (2)	14	6	1694	2.72	193	6.3
HD + 5% whey adsorbate (3)	10	6	1523	2.57	152	3.2
HD + 5% whey abdorbate + 5% rice bran filtrate (3)	11	6	1453	2.33	136	50.6
		Experiment 2				
Diet HA (1)	17	6	1779	3.51	301	54.0
Diet HA (2)	12	5	1662	3.42	188	65.4
Diet HD (1)	17	6	1718	3.29	274	2.5
Diet HD (2)	12	5	1667	4.26	253	6.6
HD + 5% rice bran filtrate (1)	17	6	1732	3.85	330	14.0
HD + alcohol ppt. ⌓ 7.5% brewer's yeast (2)	12	5	1719	3.91 ⌓ 3	217	8.7
HD + alcohol ppt. ⌓ 7.5% brewer's yeast + 5% rice bran filtrate (2)	12	5	1676	3.51 ⌓ 3	195	50.6

obtained. In the next experimental period, however, this improvement was not maintained, as the hatch of the fertile eggs dropped to 6.3%, in spite of the fact that the amount of rice bran filtrate in diet HD was increased to 10%. When diet HD was supplemented with 5% of whey adsorbate, the average hatch of the fertile eggs was no greater than that obtained with the rice bran filtrate. On the other hand, when diet HD was supplemented with both rice bran filtrate and whey adsorbate, the average hatch of the fertile eggs was 50.6%, a striking increase above that obtained when either of these supplements was fed alone. It is evident from these results, therefore, that the rice bran filtrate (antidermatosis vitamin) and the whey adsorbate (new factor) are both required for reproduction in the domestic fowl. A preliminary announcement of the role of the antidermatosis vitamin in reproduction was made by Bauernfeind and Norris ('39).

The hens fed diet HD failed to attain as good reproduction or maintain their weight as well as those fed diet HA. Whether these results were due to the fact that more of the new factor is required for reproduction and maintenance of weight than was fed, or whether diet HD is lacking in another factor, or factors, required for these purposes, cannot be determined from the evidence. Since the average egg production per hen per week was approximately the same throughout this experiment, it appears that neither the antidermatosis vitamin nor the new factor, under the conditions of the experiment, is concerned in the maintenance of egg production.

In a repetition of this work the following year using another group of hens, results were obtained which confirmed those of the first experiment. The plan of the second experiment and the results are also presented in table 4. In this experiment the average hatch of the fertile eggs of the hens fed diet HD varied from 2.5 to 6.6% as compared to 54.0 to 65.4% for the eggs of the hens fed diet HA. When diet HD was supplemented with 5% of rice bran, the average hatch of the fertile eggs was 14%. When diet HD was supplemented with an alcohol precipitate of the water extract of dried brewer's

yeast, a material found by Schumacher and Heuser ('39) to be a rich source of the new factor and reasonably free of the antidermatosis vitamin and riboflavin, the average hatch of the fertile eggs was 8.7%. The slight increase in reproduction obtained by adding either one of these supplements to diet HD is not believed to be significant. On the other hand, when diet HD was supplemented with both rice bran filtrate and the alcohol precipitate, the average hatch of the fertile eggs was 50.6%, a striking increase in reproduction above that obtained on diet HD alone or when supplemented with either one of these materials.

When the second experiment was in progress, Jukes ('39) and Woolley, Waisman and Elvehjem ('39) presented evidence strongly indicating that the antidermatosis vitamin is identical with pantothenic acid, obtained from liver by Williams, Truesdail, Weinstock, Rohrmann, Lyman and McBurney ('38). In this study the filtrate obtained by treating a water extract of rice bran with fuller's earth prevented the development of dermatosis in chicks fed diet CD and markedly increased the hatch of the fertile eggs of hens fed diet HD. The few chicks that hatched from the eggs of hens fed diet HD developed dermatosis much sooner, when fed a diet deficient in the antidermatosis vitamin, than normal chicks. This is evidence that the eggs produced by hens fed diet HD contained less antidermatosis vitamin than normal eggs. While these results are suggestive, it is impossible to conclude from them that the factor in rice bran filtrate necessary for reproduction in hens fed diet HD is identical with the antidermatosis vitamin in the sense to which it now appears restricted by the discovery of Jukes, and of Woolley and associates.

SUMMARY

In experimental work on the role of the antidermatosis vitamin in the nutrition of the mature fowl, an experimental diet deficient in the vitamin was developed by subjecting the cereal portion and the liver extract to prolonged dry heat treatment and the casein to special purification. This diet was shown to

be adequate in vitamins A, B, B_4, B_6, D, E, G, K, and the anti-encephalomalacia factor. Nicotinic acid was found either to be present in the diet or else not needed in avian nutrition.

The hen diet modified to make it adaptable for feeding chicks was found, when supplemented with the antidermatosis vitamin, to be deficient in another factor required for chick growth, not identical with any of the known vitamins. The new factor was shown to be destroyed by prolonged dry heat treatment, stable in yeast to autoclaving, water-soluble, adsorbable upon fuller's earth and present in milk, liver, yeast and fresh green grass.

Both the antidermatosis vitamin and the new factor were found to be necessary for reproduction in the domestic fowl. Supplementing the deficient diet with both these factors, however, failed to promote normal reproduction or to maintain the weight of the hens, but egg production was satisfactory. Whether these results were evidence that an insufficient quantity of the new factor was fed or that the experimental diet was deficient in another factor, or factors, required for reproduction and maintenance of weight, could not be determined from the evidence.

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A QUANTITATIVE CHEMICAL STUDY OF THE URINARY EXCRETION OF THIAMINE BY NORMAL INDIVIDUALS [1]

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(Received for publication July 14, 1939)

SEVEN FIGURES

The presence of thiamine in human urine was first demonstrated by Muckenfuss ('18) and this finding subsequently confirmed by van der Walle ('22). Recent studies have been concerned with the quantitative estimation of the vitamin in urine using both biological (Harris and Leong, '36; Roscoe, '36; Knott, '36; Helmer, '37; Leong, '37; Light, Schultz, Atkin and Cracas, '38) and chemical (Westenbrink and Goudsmit, '37 and '38 a; Karrer, '37; Ritsert, '38) methods of assay. For the latter purpose the thiochrome procedure has been used exclusively.

In the present study the thiamine content of urines from normal adults was determined by the method of Melnick and Field based upon the reaction between the vitamin and diazotized p-aminoacetophenone and described in detail elsewhere ('39 b). It has been shown to be specific. No attempt has been made in the present study to insure the excretion of an acid urine because the work of Melnick and Feld just cited showed this to be unnecessary. Since these authors have also shown

[1] The expense of this study was defrayed by grants from the Upjohn Company, Kalamazoo, and from the Rackham School of Graduate Studies, University of Michigan.

[2] Upjohn Fellow in Clinical Research, 1937–1940.

[3] Upjohn Fellow in Clinical Research, 1938–1940.

that thiamine in urine is in the free nonphosphorylated form, only estimations of the free compound were made.

The amount of thiamine in the feces was not determined in the present study. Such a determination is of questionable value since the normal fecal thiamine content appears to be constant and independent of the dietary intake, and arises practically entirely from bacterial synthesis in the lower part of the intestine (Leong, '37). This synthesized vitamin is retained in the bacterial cells and excreted with the feces (Abdel-Salaam and Leong, '38). Furthermore, no appreciable amounts of thiamine are excreted into the gut, even when large amounts of the vitamin are present in the tissues (Leong, '37).

Investigations of the factors influencing the urinary excretion of thiamine were necessary before significance could be attached to the values obtained.

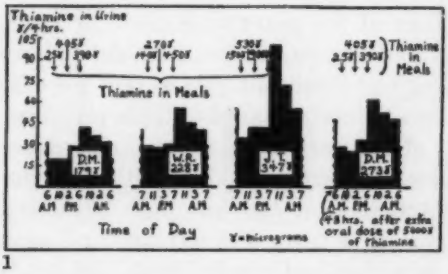
Diurnal variation in the urinary excretion of thiamine and its significance

Westenbrink and Goudsmit ('37) have reported a diurnal variation in the urinary excretion of thiamine and suggested that this may be due to a partial thiamine deficiency in the morning. Our findings indicate that the normal diurnal variation is due to an appreciable lag in excretion of the vitamin ingested in the diet and that the low values during the morning are not associated with any latent deficiency.

The urines from each of three normal male subjects, subsisting on adequate diets of calculated thiamine content,[4] were

[4] In the present report calculations of the thiamine contents of all the diets were based upon the tables compiled by Williams and Spies ('38). It is preferable to use their values in conducting balance studies with thiamine since the authors have attempted to estimate the true vitamin content with correction made for the amount of fat present. The vitamin B_1-sparing action of fat has been shown to be due to the decreased metabolic requirements for thiamine when the fat content of the diet is increased (Stirn, Arnold and Elvehjem, '39) and not to the actual presence of the vitamin in the foodstuff (Melnick and Field, '39 a). Thus, variations in ratio of fat to the non-fat constituents of each food can alter markedly the apparent thiamine content as estimated by methods of biological assay.

collected during six consecutive 4-hour periods and analyzed separately. The results obtained are presented in figure 1. The test was repeated with one of the subjects, subsisting on the same diet, but this time 48 hours subsequent to the ingestion of an extra oral dose of 5 mg. of thiamine (fig. 1). It is improbable that any latent thiamine deficiency existed since the subject now excreted an amount of thiamine, 273 micrograms, far in excess of those recorded for this individual while ingesting the constant diet (table 2). The values for each 4-hour period were now approximately 50% greater than before



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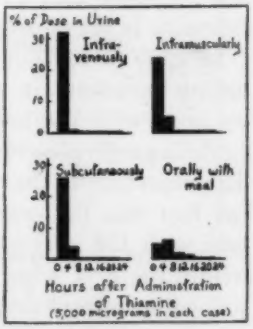

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Fig. 1 Normal diurnal variation in the urinary thiamine values.

Fig. 2 Urinary excretion of thiamine when administered parenterally and orally to the same normal subject, corrected for the basal excretion of thiamine.

with the previously observed relationship in thiamine excretion during the successive periods remaining unchanged. Also the diurnal variation observed in one subject while on a normal diet disappeared when he subsisted on a thiamine-free diet of washed polished rice, butter and dextrose.

Urinary excretion of thiamine following the administration of a test dose of extra vitamin

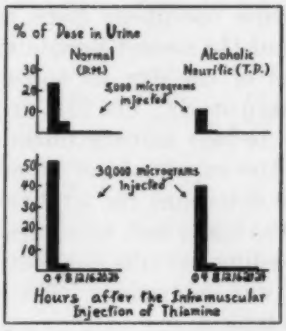
A 5 mg. test dose of the vitamin was administered both parenterally and orally to the same three subjects used in the study of the diurnal variations in thiamine excretion. In these

tests the individuals subsisted on the same diets as previously used so that corrections could be made for the amount of the vitamin normally excreted in the corresponding periods (fig. 1). The percentages of the dose recovered in the urine, partitioned and collected from one of the subjects (D.M.) over the 24-hour periods are presented in figure 2. The results with the other subjects were essentially the same. When the vitamin is given parenterally most of the total amount excreted is found in the first 4-hour sample. The greatest amount recovered followed the intravenous injection of the vitamin. A more gradual and a smaller total excretion is observed when thiamine is taken orally with a meal.

In spite of the advantage of the parenteral route in eliminating variations in rate and degree of intestinal absorption, we agree with Westenbrink and Goudsmit ('38 b) that the oral route is preferable for the administration of a single test dose. The objection to the use of the parenteral routes arises from the fact that the organism is rapidly 'flooded' with the vitamin with the major excretion after the test dose occurring within the first 4-hour period. Figure 3 illustrates that such a flooding effect occurs in avitaminotic individuals and tends to mask the difference in excretion between such persons and those normally nourished. The alcoholic neuritic excreted approximately 30% as much thiamine as the normal individual when no extra vitamin was given. When the test dose was injected, large amounts of thiamine were recovered from the urine. When the dose was increased, the percentage recovery of the extra thiamine from the urine of this patient showed relatively a much greater gain (300%) as compared with the normal (100%). The difference in thiamine excretion between these individuals was now largely eliminated. Clinical use of the oral test dose for supplementing information obtained by analyzing the usual 24-hour urine specimens has proved very valuable (Robinson, Melnick and Field, '40).

Further studies, presented in figure 4, indicate the importance of giving the oral test dose with a meal. When an aqueous

solution [5] of 5 mg. of thiamine is taken 12 hours after the last meal and 3 hours prior to breakfast, the fraction excreted in the urine is much less than when the same dose is taken with the largest meal of the day.[6] Apparently a considerable part of the vitamin taken into the fasting stomach, is destroyed prior to absorption. However, the major excretions of thiamine during the first 4-hour periods suggest that the vitamin



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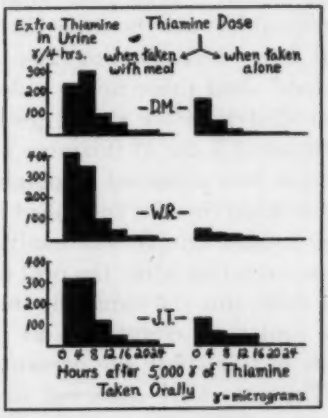

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Fig. 3 'Flooding' effect in both normal and avitaminotic subjects when the test dose of extra thiamine is administered parenterally. Correction was made for the basal excretion of thiamine.

Fig. 4 Difference in the urinary excretion of thiamine when the oral test dose of extra vitamin is taken with a meal and without a meal with the normal subject now post-absorptive. Correction was made for the basal excretion of thiamine.

is absorbed more rapidly under these conditions. Investigations of the stability of thiamine in the gastro-intestinal secretions under conditions simulating those in vivo are now in progress.

[5] In such a solution, adjusted to a pH acid to congo red and stored in the refrigerator, there will be no destruction of the vitamin for a period of at least 1 month.

[6] In these studies the subjects subsisted on the same diets used in the study of the diurnal variations (fig. 1) so that corrections could be made for the amount of the vitamin normally excreted.

The 24-hour urinary excretion of thiamine and the response to an oral test dose of extra vitamin

Fifteen normal men and ten normal women, from 25 to 35 years of age, were used in this study. The subjects were hospital staff members and their wives, subsisting on supposedly adequate diets composed of a wide variety of foods. Selection of foods during the test period was left to the choice of the subject and represented what that individual generally ate. Each subject ate the same diet for at least 2 consecutive days and two corresponding 24-hour urine specimens were collected.[1] Just prior to the collection of the second sample and immediately after the largest meal of the day an aqueous solution of 5 mg. of thiamine was taken orally. The first urine sample was analyzed to obtain the 24-hour urinary thiamine value when the diet furnished the entire supply of the vitamin. The second sample was analyzed to determine the total thiamine excretion after the oral dose was taken and, by subtracting from this the value for the preceding day, the per cent of the oral dose excreted in 24 hours was determined. The results of this study are presented in table 1.

The men were observed to excrete from 90 to 350 micrograms of thiamine per day, with the average value of 198. The latter represents an excretion of about 20% of the dietary intake. From 8 to 19% of the oral test dose was recovered from the urine, averaging 14%. The women excreted much less thiamine than the men, the values ranging from 61 to 146 micrograms of thiamine per day with an average value of 93 micrograms, or about 14% of the dietary intake. About 12% of the oral test dose was recovered from the urine on the following day.

Variations among the 24-hour urinary thiamine values obtained with these subjects cannot be attributed to differences in metabolism or in either the vitamin to calorie (Cowgill, '34) or in the thiamine to non-fat calorie ratio of the diet (Wil-

[1] The two 24-hour periods, during which the urine samples were collected, began in each case after dinner.

TABLE 1

Urinary excretion of thiamine by normal men and women before and after the oral administration of 5000 micrograms (γ) of extra vitamin

SUBJECT	WEIGHT (HEIGHT)	DIETARY THIAMINE INTAKE [1]	THIAMINE NON-FAT CALORIE RATIO	24-HOUR URINE VOLUME		THIAMINE EXCRETED IN URINE		FRACTION EXCRETED OF	
				Before oral dose	After oral dose	Before oral dose	After oral dose	Dietary intake	Oral dose [2]
males	*kg.* (*cm.*)	*γ/24 hrs.*		*cc.*	*cc.*	*γ/24 hrs.*	*γ/24 hrs.*	%	%
D.M.	74 (170)	820	0.5	900	1110	168	915	21	15
W.R.	68 (178)	860	0.5	1470	1100	228	1182	27	19
J.T.	66 (163)	1580	1.2	900	950	324	1236	21	18
D.L.	82 (183)	610	0.6	1830	1920	90	732	15	13
H.H.	77 (183)	1000	0.7	1740	2040	248	1025	25	16
H.F.	78 (173)	1130	0.9	2230	1310	237	740	21	10
C.K.	65 (175)	1130	0.8	1300	1940	171	600	15	9
H.A.H.	72 (173)	930	0.8	920	1080	146	550	16	8
K.H.	73 (183)	890	1.2	1220	1580	168	1120	19	19
J.S.	70 (170)	610	0.7	1400	1220	102	847	17	15
J.A.C.	68 (173)	?	?	1020	....	123	....	?	..
B.B.	71 (180)	?	?	1210	....	140	....	?	..
W.T.	59 (173)	?	?	730	....	350	....	?	..
R.C.	79 (183)	?	?	1510	....	201	....	?	..
J.C.	74 (190)	?	?	1730	....	269	....	?	..
Average male	72 (177)	960	0.8	1340	1430	198	895	20	14
females									
H.T.	50 (152)	750	1.0	1500	1110	95	606	13	10
F.S.	50 (157)	640	0.9	850	1200	68	500	11	9
E.L.	54 (157)	840	1.1	1030	1060	74	468	9	8
M.C.	57 (168)	840	0.8	2000	1800	80	774	10	14
K.S.	52 (152)	690	1.1	1910	1600	61	1034	9	19
D.F.	65 (163)	580	0.8	1460	800	77	800	13	14
A.H.	40 (152)	870	1.3	1080	730	146	720	17	12
E.S.	64 (168)	450	0.4	1550	1200	139	637	31	10
H.W.	47 (153)	?	?	780	810	100	726	?	13
B.L.[3]	(59) (165)	(340)	(0.5)	(1000)	(1020)	(40)	(366)	(12)	(7)
Average female	53 (158)	710	0.9	1350	1150	93	696	14	12

[1] Calculations of the thiamine contents of the diets were based upon the tables compiled by Williams and Spies ('38).

[2] Calculated by subtracting from the total thiamine, excreted after the administration of the oral test dose, the 24-hour urinary thiamine value of the preceding period and dividing the difference by 5000 micrograms.

[3] The values for this individual with respect to thiamine intake and excretion both before and after the administration of the oral test dose are so below the others that they have been eliminated in calculating the average values. This may be a case of latent deficiency.

liams and Spies, '38). The urine volume also appears to be a factor of insignificant importance in governing the urinary excretion of thiamine. Prolonged diuresis has been reported by Cowgill and associates ('30) to cause an earlier onset of vitamin B_1 deficiency in dogs subsisting on a diet inadequate in thiamine content. However, in the present study a single marked diuresis by one of the subjects, subsisting on a standardized diet but in addition forcing fluids, was not associated with any increase in the urinary thiamine values.

Further studies were carried out to establish the significance of the urinary thiamine values. Thirty 24-hour urine samples, collected from two of the male subjects over a period of 8 months, were analyzed. No attempt was made to eat the same diet during this period. The values obtained with one individual (D.M.) varied from 120 to 200 micrograms and with the other (W.R.) from 120 to 260 micrograms of thiamine. Some correlation was observed between thiamine excretion and the dietary intake. This marked variation in thiamine excretion by the same individual, however, is no longer apparent when the subject returns to the same diet for each test. This is shown by the representative data obtained with each subject and presented in table 2. Because of this reproducibility we feel that the values reported in table 1, although varying rather widely among the different individuals, are valid and do not represent any appreciable 'carry-over' effects of the previous diets. The response to the oral administration of extra vitamin is also reproducible. Large differences in the meals consumed by the subjects do not alter appreciably the characteristic response of the individuals to the oral test dose ingested along with these meals. The evening meals of two of the subjects (H.T. and J.T.), who ingested in these earlier experiments the same foods but different quantities of them, were now reversed and then followed immediately with the same oral test dose. The percentage excretions of the extra vitamin in each case were practically the same as when these subjects received the dose along with their regular meals.

In evaluating the adequacy of a patient's dietary régime, individual variation in thiamine excretion is no complicating factor. It is the minimal normal value which is of significance, 90 micrograms per 24 hours in the case of the males and 60 in the case of the female subjects (average-sized, sedentary adults). With patients giving reliable dietary histories of inadequate thiamine intake, the 24-hour urinary thiamine figures are always below these minimal normal values (Robinson, Melnick and Field, '40).

TABLE 2

Reproducibility of the values for the 24-hour urinary excretion of thiamine before and after the oral administration of extra vitamin

SUBJECT (SEX)	DATE [1] 1939	THIAMINE INTAKE		THIAMINE EXCRETION	
		Diet [2]	Oral dose	Before oral dose	After oral dose
		micrograms	*micrograms*	*micrograms*	*micrograms*
D.M.	1/30	820	5000	168	915
(♂)	3/27	820	5000	200	1002
W.R.	2/20	860	5000	198	1182
(♂)	3/20	860	5000	180	1200
J.T.	2/21	1580	5000	324	1236
(♂)	3/ 7	1580	5000	347	1212
H.T.	2/21	750	5000	95	606
(♀)	3/16	750	5000	89	561
Maximal deviation of results from the averaged values				9%	5%
Average deviation				5%	3%

[1] On the first day of the test, indicated by the recorded date, the diet alone furnished the sole source of thiamine. On the following day the 5 mg. dose of extra vitamin was taken with the subject still ingesting the same diet.

[2] Calculations of the thiamine contents of the diets were based upon the tables compiled by Williams and Spies ('38).

The response of the normal individual to inadequate thiamine intake

The diet used in this study consisted of washed polished rice, white bread, Cheddar cheese, butter, 20% cream, and dextrose, supplemented with adequate quantities of vitamin A, ascorbic acid, vitamin D and nicotinic acid. In table 3 the composition of this diet is compared with that of the normal basal diets fed these individuals. Attempts were made to keep con-

stant all variables but thiamine intake. One of the subjects (W.R.) subsisted on the experimental diet for a period of 32 days. After the twenty-second day the ration was supplemented with thiamine to give a total intake equal to that consumed in the normal basal diet. The vitamin supplement in aqueous solution was partitioned so that the total thiamine intake at each meal was exactly the same as when ingesting the normal diet. The results of this study are presented in figure 5. It will be observed that the urinary thiamine values decreased precipitously from the normal to values characteristic of avitaminotic individuals (Robinson, Melnick and Field,

TABLE 3

Comparison of the normal basal diets with the thiamine inadequate ration used in the present study

SUBJECT	DIET	THIAMINE INTAKE [1]	CALORIC INTAKE		THIAMINE NON-FAT CALORIE RATIO
			Total	Non-fat	
		micrograms	*calories*	*calories*	
W.R.	Normal basal	860	2900	1680	0.5
	Thiamine inadequate	260	2800	1670	0.16
	Inadequate + added thiamine	860	2800	1670	0.5
D.M.	Normal basal	820	2740	1700	0.5
	Thiamine inadequate	260	2800	1670	0.16

[1] Calculations of the thiamine contents of the diets were based upon the tables compiled by Williams and Spies ('38).

'40). At the time when vitamin supplementation was begun on the twenty-third day, the body stores of thiamine were obviously significantly reduced. Actually there was apparent definite clinical evidence of a deficiency characterized by aching calf muscles after progressively decreasing amounts of exercise, tenderness of calf muscles and Achilles tendons, and paresthesias of lower extremities. The low values of thiamine excretion persisted despite supplementation of the diet with the vitamin. In order to show that there were no unknown factors associated with the ingestion of the inadequate diet tending to augment the thiamine requirements, the subject

returned to his normal basal ration. The urinary thiamine values remained in the subnormal range. The per cent of the oral dose excreted in the urine at the end of the study, however, was comparable to the normal value characteristic for this individual. Apparently the thiamine stores of this individual were gradually replenished when the vitamin intake was returned to the normal control level, so that the excessive amounts normally available for excretion were not present.

In the studies by Harris and Leong ('36) the normal experimental subjects subsisted on the inadequate thiamine diet for

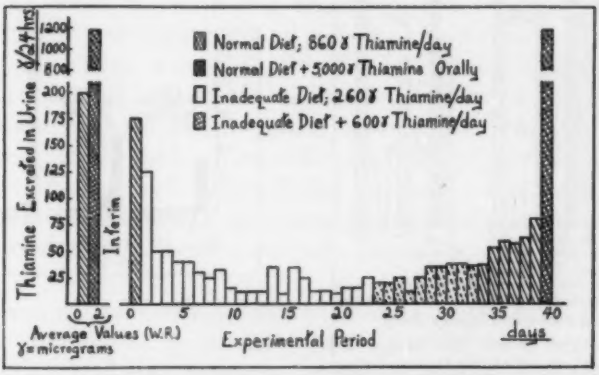
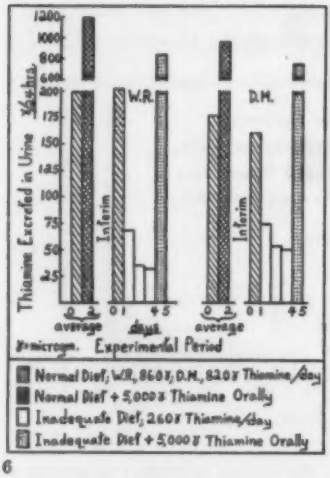


Fig. 5 Urinary excretion of thiamine by the normal individual subsisting on an inadequate thiamine diet and then on the same ration with added vitamin.

a period of only 3 days when the adequate diets were again ingested. They also observed the rapid decrease in the quantities of the vitamin excreted. However, the return to the normal values on re-alimentation was, in their studies, equally as rapid. Their findings would seem to indicate that the 24-hour urinary thiamine values reflect nothing more than the adequacy of the diet being consumed at the time of the test. However, from the results in the present study, the 24-hour values become of diagnostic importance in demonstrating objectively whether or not a true thiamine deficiency exists. A low value does not indicate irrevocably that the individual is

deficient. It may suggest simply that an inadequate diet was ingested shortly prior to and during the test. However, if the patient is given a normal diet a few days prior and during the day of the test and still excretes very small amounts of the vitamin, a deficiency may be considered to be present.

The use of an oral test dose, by minimizing the differences in available thiamine due to variations in dietary intake,



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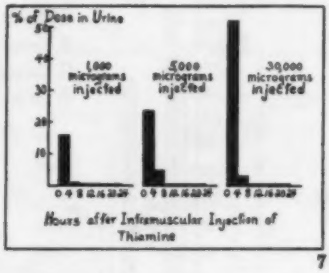
% of Dose in Urine
1,000 micrograms injected
5,000 micrograms injected
30,000 micrograms injected
Hours after Intramuscular Injection of Thiamine

7

Fig. 6 Normal urinary excretion of extra thiamine by normal subjects, subsequent to the ingestion of the oral test dose, despite the very low 24-hour urinary thiamine value preceding the test.

Fig. 7 Increase in the percentage excretion of thiamine in the urine as the amount available to the normal organism is increased. Correction was made for the basal excretion of thiamine.

should be of value in determining the degree of saturation of the body with the vitamin regardless of the adequacy of the diet being ingested at the time. When such a test is carried out, there should be available a sufficiently large excess of thiamine so that the normal organism ought to excrete a normal fraction of the test dose even though the previous 24-hour value may be low due simply to the inadequacy of the diet.

That this does take place is indicated from the studies presented in figure 6. In each case the percent of the oral dose excreted, correction having been made for the urinary thiamine value of the previous 24-hour period, was comparable to that found when the normal subject ingested an adequate diet.

It should be noted that the percentage of the ingested vitamin excreted in the urine decreases markedly when the normal intake is reduced to an inadequate level. In the study, summarized in figure 5, these values dropped from 20 to 5% of the dietary intake. That the percentage of the available vitamin, which is excreted in the urine, is a function of how great an excess is present is indicated in figure 7. In this study correction was made for the amount of thiamine excreted when the subject (D.M.) subsisted on the same normal basal diet with no extra vitamin administered (fig. 1). This response of the body to variable amounts of available thiamine may very well be the factor primarily responsible for the smaller percentage excretion of ingested vitamin in the case of the normal women subjects (table 1).

Return of the urinary thiamine values to the basal level subsequent to the daily oral administration of large quantities of extra vitamin

The procedures outlined in this report may be used to evaluate the results of thiamine therapy. Analysis of the urine at the time when a patient is receiving such therapy is worthless because flooding the organism with the vitamin, even in cases of deficiency, results in the excretion of a large amount of thiamine (fig. 3).

Two standardized subjects (D.M. and H.T., table 2) ingested daily 10 mg. of thiamine along with a normal adequate diet for a period of 23 days. This régime is comparable to the therapeutic measures to which thiamine-deficient patients are subjected. Subsequent to this period the subjects returned to the same basal diet used in the standardization of these individuals. Fully 2 weeks were required before the 24-hour uri-

nary thiamine values returned to the previous basal level. Accordingly, such a period of time should be allowed to elapse subsequent to therapy before conducting analyses of urines from treated patients. This period of 2 weeks is probably well above the minimal limit, since this study was conducted with normal subjects. Deficient individuals have in addition another pathway for the removal of the large amounts of thiamine administered, namely, the replenishment of the thiamine stores in the tissues.

Application of the observations made in the present study to problems of clinical interest

As a result of our studies of the urinary excretion of thiamine by normal individuals, several fundamental observations have been made which lend themselves to clinical application. The standards, which we have set up, are presented in table 4 and should apply to studies using procedures similar to our own.

Studies of clinical cases of thiamine deficiency (Robinson, Melnick and Field, '40) support the validity of the interpretations which have been applied to the urinary thiamine values. In our investigations of normal and clinical cases more than 120 subjects were used. In about 80% of these a close parallelism was observed between the 24-hour urinary thiamine values and the fractions of the oral test dose excreted. In the remaining 20%, with practically no exception, the responses to the administration of the oral dose were normal despite the very low 24-hour urinary thiamine values preceding the tests. This disagreement has been interpreted as indicating an inadequacy in the diet ingested immediately prior or during the test but as yet associated with no concomitant depletion of the thiamine stores of the organism. Low urinary thiamine values, subsequent to the administration of the oral test dose to patients with normal 24-hour values for the period prior to the test, were practically never observed.

TABLE 4

Standards for the evaluation of the nutritional status of adult subjects with respect to thiamine

ADULT MALE SUBJECTS		INTERPRETATIONS	ADULT FEMALE SUBJECTS	
24-hour urinary thiamine value	Fraction of oral dose excreted in 24 hours [1]		Fraction of oral dose excreted in 24 hours [1]	24-hour urinary thiamine value
micrograms	%		%	*micrograms*
above 100	7–20	Normal subject subsisting on a normal diet.	7–20	above 70
	1– 7	Subject deficient but receiving thiamine therapy during the time of the test.[2]	1– 7	
	10–20	Subject normal; diet consumed during test is inadequate or probably borderline with respect to thiamine content.	10–20	
90–100	7–10	Subject borderline with respect to thiamine stores; diet characteristically eaten by subject.	7–10	60–70
	1– 7	Subject approaching a deficiency; diet of very high thiamine content ingested during test.[3]	1– 7	
	10–20	Subject normal; diet consumed during test is inadequate with respect to thiamine content.[3]	10–20	
	7–10	Subject mildly deficient; diet may or may not	7–10	
60–90		have been adequate in thiamine content during day of test.		40–60
	1– 7	Subject deficient, diet probably adequate during day of test.	1– 7	
	10–20	Subject normal; diet consumed during test is very inadequate with respect to thiamine content.[3]	10–20	
	7–10	Subject mildly deficient; diet may or may not	7–10	
0–60		have been adequate in thiamine content during day of test.		0–40
	1– 7	Subject markedly deficient; diet may or may not have been adequate.	1– 7	

[1] Correction is made for the thiamine excreted during the preceding 24-hour period when the subject consumed the same ration but with no test dose of extra vitamin administered.

[2] It is conceivable that an individual may have an abnormally low capacity for the absorption of the vitamin, but because of the daily ingestion of a diet very rich in thiamine no deficiency occurs. Under such circumstances it then becomes possible that the 24-hour urinary thiamine value may be well within the normal range but the percentage recovery of the oral dose may be low. However, in our studies we have practically never observed such a relationship in thiamine excretion before and after the ingestion of the oral dose unless the tests were conducted with an individual deficient in thiamine and receiving therapy.

[3] It is likewise conceivable that an individual may be deficient but, because of an inability to utilize the vitamin though available, a large fraction is excreted in the urine. For example, the phosphorylation of thiamine may be assumed to occur principally in the liver (Ochoa and Peters, '38) and the vitamin has been shown to exist, in the organism for the most part in the phosphorylated state (Westenbrink and Goudsmit, '38 b; Ochoa and Peters, '38) as a coenzyme in one of the important enzyme systems in the body (Lohmann and Schuster, '37). An injury to the phosphorylating mechanism may result in the inability of the body to use the vitamin. However, the possibility of such an alteration in thiamine metabolism has never been demonstrated. Furthermore, our studies of cases of thiamine deficiency with very marked liver disease, who received thiamine intravenously over a period of several hours, have indicated that the vitamin is retained to the same extent as in patients with uncomplicated avitaminoses (Field, Robinson and Melnick, '40).

SUMMARY

The reaction between thiamine and diazotized p-aminoacetophenone in alkaline solution has been used for the chemical determination of the vitamin in urine. There is a diurnal variation in thiamine excretion due to an appreciable lag in the excretion of the vitamin subsequent to its ingestion as part of the meals. The responses of the standardized subject to both the oral and parenteral administration of a test dose of extra thiamine have indicated that the former procedure is more desirable for evaluating the nutritional status of the individual with respect to this vitamin. The average 24-hour urinary thiamine values of fifteen normal men and nine normal women were 198 and 93 micrograms respectively; 14% of the oral test dose of 5 mg. of extra vitamin were excreted by the men during the first 24-hour period following ingestion and 12% by the women. Variations in these values among the normal subjects were not related to differences in urine volume, in metabolism or in either the vitamin to calorie or thiamine to non-fat calorie ratio of the diet. Urinary thiamine values of normal individuals ingesting a constant diet are reproducible. During the production of an experimental thiamine deficiency the 24-hour urinary values drop precipitously to the avitaminotic range but rise slowly during recovery. The response of the subject to the oral test dose of extra thiamine is governed by the nutritional status of the individual and is independent of the adequacy of the diet consumed at the time of the test. The percentage of the available vitamin, which is excreted in the urine, is a function of how great an excess is present. Subsequent to flooding the organism with thiamine a period of 2 weeks is required to elapse before the urinary values return to the normal basal level. Standards are given for the interpretation of the urinary thiamine values in the diagnosis of thiamine deficiency.

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THE ISOLATION OF DESIRED QUALITIES OF RADIANT ENERGY FOR BIOLOGICAL EXPERIMENTATION [1]

LOYD A. JONES AND CLIFTON TUTTLE

SIX FIGURES

(Received for publication April 3, 1939)

Equipment for the isolation of a number of fairly narrow spectral regions of continuous radiation in the infra-red, visual and ultra-violet was described by one of us in a previous paper (Jones, '29). The source of radiation in this equipment was a carbon arc, and various filters were used beneath the arc to absorb unwanted regions. An area about 1 foot by 1½ feet was radiated to energy levels ranging from 0.003 calories/cm.²/min. in the ultra-violet to 0.122 calories/cm.²/min. in the infra-red.

The findings of Luce-Clausen who made a study of the biological action of these radiation bands on the rat (Luce-Clausen, '29) indicated that further experiments particularly designed to compare the near infra-red and visual regions might prove significant. The additional requirements of these proposed experiments as set forth by Luce-Clausen were twofold: first, that considerably larger areas should be radiated to provide for simultaneous treatment of a larger number of animals to place the experiment upon a sound statistical basis; second, that the equipment should be capable of either continuous operation or at least of operation for a much longer time than was possible in the first series of experiments.

[1] Communication no. 720 from the Kodak Research Laboratories, Rochester, New York.

A table space of about 3 feet by 9 inches is necessary for the requisite number of animals to be exposed in each spectral region. It is desirable to maintain over this entire area an energy level, continuous in each band, of the order of 0.05 calories/cm.[2]/min.

Choice of light source. Because a light source emitting a continuous spectrum was essential, gaseous discharge arcs were eliminated.[2] The frequent attention required for the operation of carbon arc lamps makes these impractical for the present purpose.

The only source available was the incandescent tungsten lamp. From the point of view of ease of maintenance, cleanliness and economy, the incandescent lamp is thoroughly satisfactory. As a radiator in the near infra-red it is also good, but as a source of a considerable amount of energy in the visual it leaves much to be desired, especially when this visual radiation must be freed from infra-red. Figure 1 shows the spectral energy distribution of a tungsten lamp at 3000° K. (1000-hour life rating). Only about 14% of the total energy emitted by this lamp lies in the visual and most of the remainder in the infra-red.

Choice of filter materials. In the previous experiment (Jones, '29) there was much flexibility in the choice of filter constituents because the area to be radiated was small and the time of radiation was short. The best absorbers of the long wavelengths are liquids. In the present experiment which had to be carried on continuously over larger areas, liquid filters were out of the question for obvious reasons. The problem of isolating the two spectral regions consists then in selecting the most efficient filter materials for the available solids, such as special glasses and dyed gelatins.

Isolation of the visual. Probably the best available material for the separation of the infra-red from visual radiation is Corning Aklo glass. The curve in figure 2 shows the optical

[2] The fluorescent tubes excited by gaseous discharge arcs which have recently become commercially available emit nearly continuous radiation in the visual spectrum and might have proved useful for this experiment had they been available at the time.

density of the medium grade of this glass plotted against the wavelength. It should be noted that there is a marked transmission band at 500 mμ where the density is 0.4 (transmittance about 40%) and that toward the longer wavelengths the density rises sharply to 3.0 (transmittance 0.1%) at 760 mμ. The density remains above 3.0 throughout the near infra-red region but decreases again for wavelengths longer than 1.6 μ.

With this glass alone covering the tungsten source, there would be an appreciable amount of long wavelength energy transmitted. An additional source of very long wavelength radiation is the Aklo glass itself which becomes heated by virtue of the energy which it has absorbed and may radiate strongly unless it is effectively cooled.

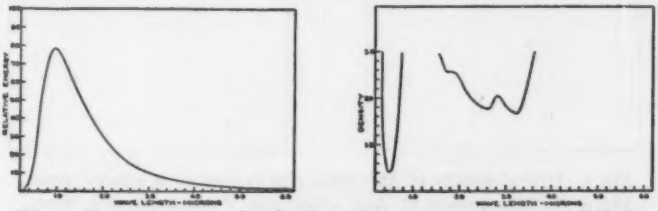

Fig. 1 Spectral energy distribution of a tungsten lamp at 3000° K.
Fig. 2 Optical density of Corning 'Medium' Aklo glass.

Ordinary double-thickness window glass is an effective shield from the far infra-red radiation. With two layers of such glass separated from each other and from the Aklo glass by air spaces, much of the unwanted long wavelength energy originating in or transmitted by the Aklo glass may be prevented from reaching the working plane.

The absorption characteristic of the combination filter (Aklo plus two layers of window glass) is shown in figure 3.

Isolation of far infra-red. Preliminary experiments showed that there would be great difficulty in eliminating from the visual radiation all of the extreme long wavelength infra-red. When the combination filter just described was exposed for any considerable length of time to radiation, convection and conduction of heat from the requisite number of high-wattage

tungsten sources, there was a rise in temperature of the lowest layer of window glass. Even a high velocity stream of air forced between the glass layers failed to prevent this temperature rise altogether. Had it been economically feasible to cool the lowest plate by a stream of water, the difficulty might have been overcome, but there seemed to be no effective and practical alternative.

This secondary radiation has a maximum in the neighborhood of 10 μ, far beyond the regions with which this experiment was designed to deal. Nevertheless, there seems to be no justification for assuming a priori that this radiation has no biological effect.

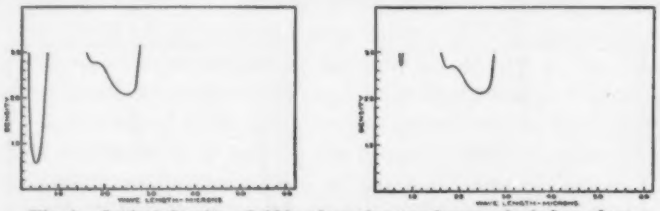

Fig. 3 Optical density of Aklo glass plus two layers of window glass.
Fig. 4 Optical density of Aklo glass plus Wratten no. 88A filter.

It was decided, therefore, that lacking the ability to achieve purity of the visible radiation, a simultaneous experiment would be carried out to test the effect of long-infra-red radiation alone. For this purpose an identical installation was made, using Aklo glass combined with a filter (Wratten no. 88A coated on glass) which completely absorbed the visible region transmitted by the Aklo glass. The density-wavelength characteristic of this combination is drawn in figure 4. It will be seen that the ultra-violet, visible and near infra-red are completely eliminated. There is a small transmission band at approximately 2.4 μ but the maximum transmission here is less than 1%. By using a few tungsten lamps mounted over the compound filter, it is quite easy to maintain the energy level at a value appreciably above the amount of long wavelength impurity which is unavoidably present along with the visual.

Isolation of the near infra-red. The third quality of radiant energy that was desired for this experiment may be referred to as the near infra-red. This was isolated by using a Corning glass filter no. 585 in combination with the infra-red (Wratten 88A) filter previously referred to. The density wavelength characteristic for this combination is shown in figure 5, in which it is seen that there is a double transmission band lying between 0.75 and 2.8 μ. There is a small band at about 4.0 but here the transmission is less than 1%.

Arrangement of the equipment. Four different chambers separated from one another by partitions were prepared for the radiation experiment. The actual setup is made clear by

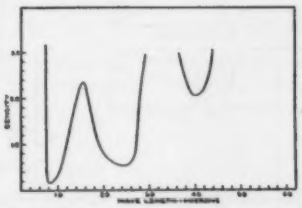

Fig. 5 Optical density of Corning no. 585 glass plus Wratten no. 88A filter.

reference to figure 6, which shows a scale plan of the whole and typical elevations of each room. Room I was kept in total darkness for the duration of the experiment and an effort was made to remove all sources of radiation from the working table where a control group of animals was kept. Rooms II, III and IV were the near infra-red, the visual and the far infra-red rooms, respectively. The arrangement in each was the same except for differences in filters and lamp wattages.

The rat cages, 10-inch cubicles of wire mesh, were arranged on tables immediately beneath the horizontal filters. The filters were framed in metal sash, as shown in the insert in the figure. Above the filters is the lamp chamber. The upper and lower filters formed the top and bottom of a duct through which air was pumped at a velocity of 700 feet per minute. Outside air was drawn into the duct through cleaning filters.

The air stream passed consecutively through the filters in each of the four rooms. A portion of the air was diverted through the lamp chamber to help remove heat dissipated by the lamps.

In room II, because of the fact that incandescent tungsten emits large amounts of energy in the region transmitted by the filter, it was only necessary to use twenty 300-watt lamps in order to obtain the desired level of near infra-red radiation. The filter materials do not absorb in regions where large quan-

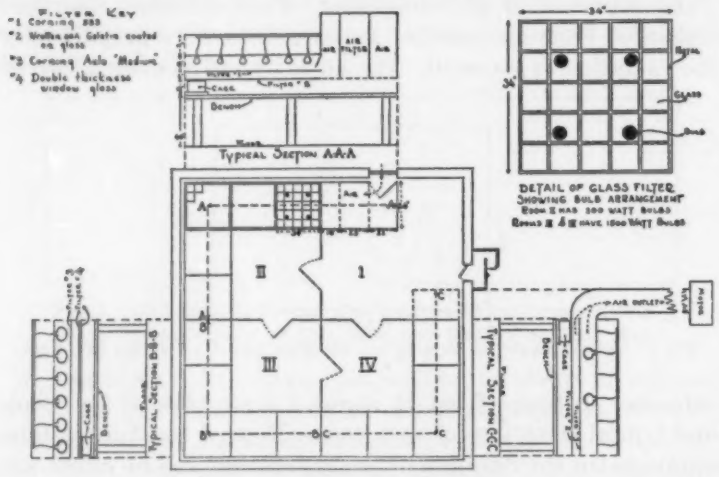

Fig. 6 Layout of radiation chambers

tities of energy are emitted and consequently they do not heat up appreciably. Hence, there is no problem of far infra-red impurity in room II.

In the visible radiation room III, twenty 1500-watt lamps were used and even with this amount of lighting the energy level was lower than was desired. Here it was that the heating of the filters gave rise to large amounts of far infra-red.

In room IV it was found that the air passing through the ducts of rooms II and III had become heated to such an extent that the filters were warmed almost to a sufficient tempera-

ture to radiate the desired amount of the far infra-red. This was particularly true during the summer when the incoming air was already warm. A few 1500-watt lamps were installed in the lamp chamber of room IV to make up the deficit in far infra-red which occurred during the winter.

An ambient temperature of 75° F. was maintained in all of the rooms throughout the course of the experiment by means of a York refrigeration unit. The air was conducted down into each room by metal pipes connected overhead with the fan of the refrigeration unit.

TABLE 1

Radiation in rooms II, III and IV in gm. cal./min./cm.²

ELAPSED TIME	ROOM NO. II G 585 + 88A		ROOM NO. III [1] Aklo + two window glass		ROOM NO. IV Aklo + 88A
	Near infra-red	Secondary	Visual	Secondary	Secondary
minutes					
Start	0.065	0.0	0.02	0.0	0.0
5	0.1	0.0	0.025	0.0	0.002
10	0.1	0.0	0.032	0.0	0.075
15	0.1	0.0	0.028	0.0	0.11
20	0.10	0.005	0.030	0.0	0.14
25	0.10	0.005	0.027	0.0	0.15
30	0.10	0.005	0.031	0.005	0.15
35	0.10	0.006	0.029	0.01	0.155
40	0.10	0.006	0.030	0.015	0.160
45	0.10	0.006	0.030	0.025	0.160
60	0.10	0.010	0.03	0.05	....
360	0.09	0.01	0.029	0.055	0.16

[1] The visually determined illumination in room III was approximately 300 foot candles.

Energy measurements and data. Seventeen-element Moll thermopiles were permanently installed in each of the rooms. These were calibrated with a standard Nernst burner. Thermoelectric output was measured with a Rawson 0 to 0.5 millivoltmeter. Connections were made through individual switches to a central reading station so that frequent checks on the operation of the equipment could be readily made.

A typical set of data is given in table 1.

Each thermopile of course reads total energy and does not discriminate between secondary and desired radiation. The secondary radiation increases steadily as the filters warm up, usually reaching a steady state after about an hour of operation. The determination of the amount of secondary present is made by interposing a sheet of the ordinary window glass over the thermopile and taking a reading before the glass has become warmed appreciably above room temperature. The readings in all the rooms are fairly consistent with the exception of the apparent increase in the visual which continues for the first 20 minutes. A possible explanation for this effect is the gradual removal of moisture on the filters by the warm stream of air. During the hottest days of summer there was some increase in secondary radiation in all rooms.

The equipment was turned on and off by a Sangamo time switch and operated for a 12-hour period each day from October, 1936 to April, 1938. During that time the only attention required was replacements of the lamps at 10-week intervals.

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THE DETERMINATION OF ASCORBIC ACID IN COMMERCIAL MILKS [1]

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(Received for publication August 7, 1939)

Milk as a source of vitamin C has attracted considerable attention recently. Holmes, Tripp, Woelffer and Satterfield ('39) have reviewed available data and have studied the breed and seasonal variations in the ascorbic acid content of certified milk from Guernseys and Holsteins. Our interest lies in the ascorbic acid content of regular commercial samples of milk as related to the quantity present in the milk at the time of milking.

EXPERIMENTAL PART

The procedure described by Mindlin and Butler ('38) which involves the use of the Evelyn photoelectric colorimeter ('36) for the determination of ascorbic acid in blood plasma was modified so as to be applicable to milk.

The oxidation of ascorbic acid other than by 2, 6,-dichlorophenolindophenol even in the presence of added copper is prevented by the use of a solution developed by Willberg ('38) which consists of sodium chloride and oxalic acid. It was modified by us to contain also metaphosphoric acid with the result that a clearer serum is obtained and the formation of a precipitate in the colorimeter tube is prevented when the dye-acetate solution is added. Loss of reducing activity is prevented in the filtrate for 16 hours if exposure to direct sunlight or strong illumination is avoided.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The volume of milk serum and quantity of dye-acetate solution are so controlled that the usual concentration of vitamin present reacts almost instantaneously. Under these same conditions the interfering substances formed in the milk after hydrogen sulfide treatment react at a slower rate, thus enabling their interference to be largely eliminated by extrapolation to zero time.

Among the interfering substances which are formed upon treatment of the milk with hydrogen sulfide, no doubt one of them is reduced riboflavin. Experimentation with ascorbic acid-riboflavin solutions under similar conditions indicates this to be the case. Apparently more riboflavin is reduced, the longer the milk serum remains in contact with the reductant, 2 hours being the average time when the effect first becomes noticeable. Fortunately, the reduced riboflavin and other substances react more slowly than ascorbic acid under the conditions used and therefore this interference is largely eliminated by extrapolation. It is to be noted that a titrimetric procedure employed on a milk serum which has been treated with hydrogen sulfide long enough to reduce the dehydroascorbic acid will invariably give results which are too high and the error involved will be dependent upon two factors—the time during which the serum has been left in contact with hydrogen sulfide and the time actually employed for the completion of the titration. Hence the use of the colorimeter or some similar instrument is essential for the determination of the dehydroascorbic acid if the effect of other reductants formed under the conditions needed to reduce the dehydroascorbic acid is to be largely eliminated.

The apparatus is identical with that used by Mindlin and Butler ('38) as is the 2, 6,-dichlorophenolindophenol, sodium acetate and indophenol acetate solutions. The potassium oxalate, cyanide and metaphosphoric acid solutions are, however, replaced by a modified Willberg ('38) reagent which is prepared accurately in order to insure the proper pH in the colorimeter tube, by dissolving in 100 ml. of glass-distilled water 0.6 gm. $H_2C_2O_4 \cdot 2H_2O$, 4.8 gm. NaCl C.P. and 6.5 gm.

HPO_3 (reagent, grade, sticks). This reagent is stable for 1 week at 15 to 20°C. and remains at constant pH.

Determination of ascorbic acid. In the absence of strong light 25 ml. of milk are pipetted into a 125 ml. Erlenmeyer flask containing 75 ml. of modified Willberg reagent. The protein precipitate is removed by filtering through paper of quality similar to Whatman 42.

Five milliliters of the filtrate are measured into a colorimeter tube and 10 ml. of the indophenol-acetate solution are added. The contents are stirred and read immediately.

Since it is not always possible to obtain a filtrate that is crystal-clear, it is recommended that the suggestion of Bessey ('38) be used; that is, a small crystal of ascorbic acid is added after the original reading has been made whereupon the correction due to the prevalent turbidity is determined. Thus the true reading is equal to the original reading plus 100 minus the reading after the crystal of ascorbic acid has been added.

Determination of dehydroascorbic acid. After the addition of a few drops of dibutyl phthalate to prevent foaming, hydrogen sulfide is bubbled through the remainder of the filtrate for 5 minutes. The tightly stoppered flask is allowed to stand for 8 hours in the dark at room temperature. The hydrogen sulfide is removed by passing a vigorous stream of wet oxygen-free nitrogen through the filtrate for 30 to 45 minutes. In order to remove the elemental sulfur and the dibutyl phthalate, the contents of the flask are filtered through paper of quality similar to Whatman 42.

Five milliliters of the filtrate are measured into one of the colorimeter tubes. With a simultaneous start of a stop watch, 10 ml. of the dye-acetate solution are blown into the tube and the galvanometer readings at 15, 30, 45, 60, 90 and 120 seconds are recorded. After the one hundred and twentieth second a few crystals of ascorbic acid are added and all these readings are corrected in the manner already described for the slight amount of turbidity that invariably seems to be present. The logarithms of the difference between

100 minus the galvanometer readings plotted against time and extrapolated to zero time will give the most accurate galvanometer reading due to the ascorbic acid plus the dehydroascorbic acid.

We have found that under the conditions described K has a value of 0.166 ± 0.003 for different lots of indophenol (practical). It is to be noted that when determining this value it is essential to have the same final pH in the colorimeter tubes as that obtained when a sample of milk is substituted for the standard ascorbic acid solution. This may readily be accomplished if the standard ascorbic acid solution employed is made by diluting three parts of modified Willberg reagent with one part of water. Other details and calculations have already been described by Mindlin and Butler ('38) and Bessey ('38). Typical recovery experiments have demonstrated a per cent recovery from 94 to 98.

DISCUSSION

Knight, Dutcher and Guerrant ('39) have shown conclusively that there is no adsorption of ascorbic acid on the precipitated proteins and are of the opinion that as the milk leaves the udder of the cow, its ascorbic acid is all in the reduced form. We have confirmed the latter point and have noted that, if the milk is immediately pipetted into the modified Willberg reagent directly after the milking machine is detached from the udder of the cow, treatment with hydrogen sulfide is unnecessary as no significant oxidation occurred during the milking operation and that the reagent prevented any further oxidation until the analysis could be completed.

Results of determinations by method presented. Recorded in table 1 are the analyses of milks of Holstein, Brown Swiss, Jersey and Guernsey cows in the regular University of Wisconsin herd. These analyses were made in January when the animals were on winter feed. The samples were secured immediately after the machine was detached from the udder and pipetted directly into the Willberg reagent. The samples were collected on 3 consecutive days during the afternoon

TABLE 1

Daily variations in the ascorbic acid content of milk from individual cows taken directly after milking

HOLSTEIN (A)[1]	ASCORBIC ACID PER LITER			PM MILKING POUNDS MILK
	1st day	2nd day	3rd day	
	mg.	mg.	mg.	
3	14.6	15.3	14.7	20
7	21.2	21.3	21.5	14
9	26.2	25.0	25.7	15
10	18.5	....	18.9	9
11	25.6	23.3	29.2	24
12	17.0	18.4	16.7	58
18	23.2	18.6	18.3	18
29	21.3	21.7	19.9	6
Average	20.9	20.5	20.6	
HOLSTEIN (B)[1]				
52	18.8	19.5	19.1	5
58	28.6	28.4	23.6	7
63	23.9	23.0	23.6	10
64	17.6	18.6	18.6	8
65	21.7	23.0	21.2	14
67	23.0	25.1	25.1	21
83	22.1	17.1	19.1	8
84	22.0	21.3	22.4	8
91	19.9	20.0	20.5	17
Average	21.7	21.6	21.7	
BROWN SWISS				
805	23.0	22.0	23.6	12
811	28.6	26.6	28.6	23
815	20.0	18.6	19.1	19
814	27.5	27.5	27.4	11
822	23.6	23.3	23.2	7
827	26.4	26.8	25.6	6
834	28.0	29.3	28.6	8
839	25.8	26.2	25.1	11
840	....	20.0	29.2	9
Average	25.3	24.4	25.6	
JERSEY				
608	21.0	20.8	20.8	7
621	23.6	24.4	20.0	6
623	31.2	29.6	29.0	14
639	15.4	16.7	17.6	7
640	31.8	29.4	27.8	10
656	18.8	22.4	21.6	8
660	22.7	24.8	22.9	10
661	23.7	20.2	25.1	14
Average	23.5	23.5	23.1	
GUERNSEY				
404	22.5	21.6	22.0	8
412	23.3	22.0	23.6	20
417	22.7	22.7	24.2	13
421	21.7	24.0	23.3	17
424	19.2	19.6	20.0	13
427	27.5	29.6	29.6	9
428	24.4	23.9	25.2	11
432	24.4	24.2	24.8	10
437	18.6	16.8	17.7	9
438	21.7	22.7	23.3	13
441	22.5	24.8	24.0	13
447	20.5	22.1	21.0	7
450	18.8	20.4	21.2	10
455	18.0	18.0	18.0	15
457	20.8	20.0	22.7	16
415	26.6	27.2	27.5	11
Average	22.2	22.4	23.0	

[1] (A) group on winter feed the year round. (B) group had summer pasture available yearly.

milking. It will be noted that the daily values for the ascorbic acid are very constant for any individual animal, that the quantity of ascorbic acid produced per liter varies significantly with each animal and that a high producer does not necessarily give less ascorbic acid per liter than a low producer.

It is further observed that milk from the Brown Swiss herd contained the most ascorbic acid per liter followed by Jersey, Guernsey and Holstein, respectively, and that the magnitude of the figures for the Guernsey and Holsteins are in close agreement with those reported by Holmes, Tripp, Woelffer and Satterfield ('39).

Samples of raw, certified, certified Guernsey and certified vitamin D milks were collected at the different dairies throughout the city of Madison. These milks (table 2) on the average are only a little below the fresh milks as recorded in table 1, indicating that commercial raw and certified milks as delivered to the consumer lose only a small amount of their antiscorbutic potency. Likewise, samples of commercial pasteurized milks were collected and analyzed. On an average they contained only about one-half as much ascorbic acid as fresh raw milks and significantly less ascorbic acid than the commercial unpasteurized milks. These results are recorded in table 3.

In both the pasteurized and unpasteurized milks the amount of dehydroascorbic acid is less than the ascorbic acid. The average percentage of dehydroascorbic acid present in the pasteurized milks makes up less of the total than in the case of unpasteurized milks. This is probably indicative of the tendency of pasteurization to form more dehydroascorbic acid which, once formed, is more readily destroyed.

Commercial pasteurized vitamin D milks in which the potency has been increased by incorporating vitamin D concentrates directly into the milk by homogenization fall below the average value of ordinary pasteurized milks in respect to the ascorbic acid content (table 4). On the other hand, if the vitamin D content has been increased by feeding the animal irradiated yeast, the antiscorbutic property falls on a par with

TABLE 2

Ascorbic acid content of commercial raw milks

COMMERCIAL MILK	ASCORBIC ACID PER LITER		
	Reduced	Oxidized	Total
	mg	*mg*	*mg*
Grade A raw	7.9	8.8	16.7
Certified dairy 1	13.1	6.3	19.4
Certified dairy 2	13.7	4.8	18.5
Certified dairy 3	15.1	4.0	19.1
Certified dairy 4	12.8	8.8	21.6
Grade A Guernsey raw	7.1	13.0	20.1
Certified Guernsey dairy 1	8.2	5.0	13.2
Certified Guernsey dairy 2	16.2	2.5	18.7
Certified vitamin D from C.L.O. (1)	7.9	6.0	13.9
Certified vitamin D from C.L.O. (2)	7.9	4.2	12.1
Average	10.9	6.3	17.3

TABLE 3

Ascorbic acid content of commercial pasteurized milks

PASTEURIZED COMMERCIAL MILK	ASCORBIC ACID PER LITER		
	Reduced	Oxidized	Total
	mg.	*mg.*	*mg.*
Guernsey dairy 1	3.8	1.8	5.6
Guernsey dairy 2	14.0	2.6	16.6
Guernsey dairy 3	7.8	8.8	16.6
Milk dairy 1	5.7	3.8	9.5
Milk dairy 2	14.0	1.1	15.1
Milk dairy 3	10.1	3.4	13.4
Milk dairy 4	12.4	5.3	17.7
Milk dairy 5	6.2	3.2	9.4
Grade A dairy 1	1.9	5.5	7.4
Grade A dairy 2	14.0	1.1	15.1
Average	8.9	3.5	12.6

TABLE 4

Ascorbic acid content of commercial pasteurized and modified milks

PASTEURIZED COMMERCIAL MILK	ASCORBIC ACID PER LITER		
	Reduced	Oxidized	Total
Vitamin D grade A (irradiated ergosterol)	3.7	3.7	7.4
Vitamin D (irradiated ergosterol added)	4.1	5.7	9.8
Vitamin D (feeding irradiated yeast)	6.9	6.8	13.7
Homogenized vitamin D (feeding irradiated yeast)	1.7	9.4	11.1
Mineral modified	2.7	5.4	8.1

the average value for commercial pasteurized milks. This would indicate that homogenization tends to destroy ascorbic acid.

Mineral modified milk is low in ascorbic acid and this is doubtless to be expected considering the treatment to which the milk must be subjected.

SUMMARY

A convenient method for the determination of ascorbic and dehydroascorbic acid in raw and commercially pasteurized milks has been described. The photoelectric colorimeter makes possible the elimination of many of the interfering substances which are formed when the milk is treated with hydrogen sulfide. Among these substances is reduced riboflavin.

The influence of breed as well as milk production has been observed as affecting the quantity of ascorbic acid produced. No daily variation in the milk of individual cows was observed.

It was found that commercial raw milks contained an antiscorbutic potency which was only slightly less than fresh raw milks and that pasteurized milks on the average contained only one-half the latter potency. Mineral modification and homogenization apparently have a destructive effect on ascorbic acid.

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MEAD JOHNSON & COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1940 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute at New Orleans on March 13, 1940.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more parties. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1939 must be in the hands of the Secretary by January 5th, 1940. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

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2-Methyl-1,4-Naphthoquinone

A Compound Exhibiting Vitamin K Activity

Recent publications from several laboratories[1,2,3,4] have shown that 2-methyl-1,4-naphthoquinone exhibits a high degree of vitamin K (anti-hemorrhagic) activity in the chick. Many investigators are of the opinion that its activity is as great or greater than that of vitamin K_1.[1,2,3,6]

Preliminary clinical tests with this compound have been reported as successful in small dosage.[5] It has been suggested[2] that in view of the high vitamin K activity of this compound and the lack of a suitable standard, 2-methyl-1,4-naphthoquinone be adopted as a basic standard for the assay of vitamin K.

General Biochemicals' 2-methyl-1,4-naphthoquinone in crystalline form is available in bulk quantities for clinical and experimental research. Upon request we shall be pleased to send a complete list of our biochemicals.

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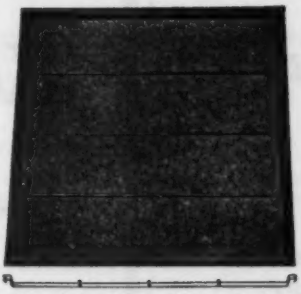

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